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STUDIES IN MICROSCOPICAL SCIENCE.

STUDIES IN

MICROSCOPICAL SCIENCE,

EDITED BY

ARTHUR C. COLE, F.R.M.S.

VOL. II.

WITH THIRTY-SIX LITHOGRAPHED PLATES.

LONDON:
BAILLIÈRE, TINDALL, AND COX,
20 KING WILLIAM STREET, STRAND, W.C.
1884.

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PREFACE.

The first volume of the "STUDIES IN MICROSCOPICAL SCIENCE" consisted of a series of Essays which, though each was complete in itself, had little connection with each other. In the present volume an attempt has been made, in the Sections devoted, respectively, to Animal and Botanical Histology, to work out a special subject in so far as the series of twelve short essays in each section admitted of such treatment. My best thanks are due to Mr. W. FEARNLEY, who contributed the Section in Animal Histology; to Mr. DAVID HOUSTON, F.L.S., F.R.M.S., Lecturer on Botany and Biology at the Birkbeck Institution, who undertook the Botanical Section. Both these gentlemen kindly contributed to the "POPULAR STUDIES," in which Section also I was greatly assisted by Mr. FREDERICK GREENING, Lecturer on Animal Morphology and Histology at the Birkbeck Institution. Mr. Fearnley rendered most valuable assistance in respect of the "METHODS OF MICROSCOPICAL RESEARCH;" whilst the very elaborate and beautiful drawings from the preparations were contributed by Mr. EDWARD T. DRAPER, to whom I offer my warmest acknowledgments. The preparations were made by my son, Mr. MARTIN J. COLE (Instructor in Practical Microscopy at the Birkbeck Institution), and myself. I desire to express my grateful sense of the very kind and appreciative notices and reviews accorded to the "STUDIES" by the Editors of the "JOURNAL OF THE ROYAL MICROSCOPICAL SOCIETY," "SCIENCE GOSSIP," "ILLUSTRATED SCIENCE MONTHLY," "THE MICROSCOPICAL NEWS," and numerous other Journals in this country and America.

ARTHUR C. COLE.

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STUDIES IN MICROSCOPICAL SCIENCE.

SECTION I.—ANIMAL HISTOLOGY.

CHAPTER I.

THE MORPHOLOGY OF THE CELL.

Although the term *histology*[1] literally signifies, a discourse about tissues in general, its meaning in natural science is now limited to a consideration of only the minute details of structure. Histology then, is pre-eminently a microscopical science; and animal histology is concerned exclusively in the investigation of animal tissues.

As in physical science the chemist builds up his system of philosophy out of atoms endowed with energy, so the histologist commences with his elements,—cells imbued with life. A distinction must be drawn between anatomical and physiological histology; the former deals with the shapes of cells and their relation to each other, the latter treats of their functional activity or life. It is only with anatomical histology that we are at present concerned, and our first inquiry must be confined to the ultimate element from which all tissues arise or are built up, and which has been termed the *morphological unit* or *cell*.

THE CELL THEORY.—Since the discovery of the cellular nature of animal tissues by SCHWANN[2], in 1835, there has been much diversity of opinion as to the true nature of the morphological unit. Some have asserted that it consists of a minute mass of indifferentiated protoplasm; that is to say, protoplasm in its most elementary condition, without any sign of structure,—not even a separation into an outer dense and an inner soft layer; others have taken up this primordial particle and have held it to be the ultimate condition from whence every morphological unit derives its existence, and, when it is in this primitive state, have considered that it deserved some special name, wherewith to distinguish it from all other things, and the *cytode*[3], as it was called, ranked as a sort of histological Adam.

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[1]Gr. ἱστος, web, tissue; and λόγος, discourse. Also written *Histiology*, from Gr. ἱστίον, tissue.

[2]*Mikroskopische Untersuchungen*, Sydenham Society's translation, London, 1847, p. 165.

[3]From the Greek for cell, κύτος; the term *cell* is derived from the Latin *cella*, akin to *celare*, to hide, conceal, hence a small closed cavity or hollow place.

But this varying nomenclature came to be only confusing, and the name *cell* once more regained its ascendancy. But it was found that as the history of the lower forms of animal life were studied and revealed, that they were nothing more nor less than single cells, it was still an open question what the cell essentially consists of. Hypotheses of their precipitation, and subsequent modification were not wanting to account for their existence. Most observers, however, agreed that the outer part, which is usually firmer in texture, was not essential, but only the result of development, and the cell-wall ceased to trouble naturalists. But, within the cell, there often exists a denser portion, usually of spherical form, and this *nucleus*, and its contained *nucleolus*, were long looked upon by some observers as the necessary origins of the cell; their conclusions being based upon the fact, that when nucleated cells multiply they generally do so by a division through the nucleus, and greater weight was attached to this, inasmuch as in all previously observed cases of sexual generation those of the nucleus heralded all subsequent changes. Moreover, amongst the unicellular animals, known as *Infusoria*, so active do the nucleus and nucleolus become, that a specialisation of function, the function of reproduction by sexual generation, was assigned to them. The importance of the nucleus to the cell falls to the ground, however, when we come to consider that there is a legion of forms which do entirely without a nucleus throughout the whole of their lives, and that, indeed, this distinction of nucleated and non-nucleated cells has been taken advantage of in a natural classification of these lowly organisms, which thus admit of a division into *Monera*[1] and *Endoplastica*.[2] Superadded to this there is the incontestible fact that nucleated cells, even amongst the highest order of animated beings, often subdivide in their development, independently of their nuclei.

From what has been stated, it will be clear that all attempts at a nomenclature which seeks to designate any single definite thing as a cell, must of necessity be futile, for the morphological unit varies, not only in its nature from the very commencement, but in the course of its individual development. Thus, the simple mass of undifferentiated protoplasm known as *Protamœba*, originates as *Protamœba*, and often remains a fixed type; but it may change, it may develop a nucleus and contractile vesicle, and become an *Amœba*. *Amœba* itself may reproduce young *Amœbæ*, which do not differ in any way from the parent organism, and which grow thereafter, but in size only. Other cases commence as *Amœbæ*, but subsequently develop into more complex forms, *e.g.*, *Hydra*.

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[1]Gr. μονός, single, an order of *Protozoa*, embracing those forms whose bodies consist of simple undifferentiated protoplasm.

[2]Gr. ἔνδον, within; πλαστός, formed; nucleated *Protozoa*.

Each cell of the adult *Hydra* assumes a columnar, or rather a cuboid shape, is nucleated, and possesses an outer membranous envelope, sometimes only recognisable as a denser hyaline portion.

Amongst the higher animals, most of the tissues of the body, save those of certain glandular organs, are composed of aggregates of nucleated cells with well-defined cell-walls. In the course of growth, some of these cells are cast off from the economy, as mere cell-walls, their contents have vanished, and they are no longer of any use to the body; they exercise a protective function to the cells beneath them for a time, and are thereafter discarded; familiar examples of such cells may be seen all over the general surface of the body; they may be readily obtained from the region of the scalp in man, whence their detached elements are known as "scurf," in the external ear, near the roots of the nails, and the transitional or red portion of the lips they are most noticeably abundant, and can easily be procured for examination; in certain local and temporary irritations, such as chaps and blisters they are sometimes too evidently manifest, and in chronically diseased conditions, as in psoriasis and scurvy, they are painfully present.

But there are other cells in the body which also ultimately lose their nuclei and cell-contents, but, in so doing, accumulate an amount of matter, in their cell-walls, upon the passive mechanical properties of which their physiological functions depend. The enamel fibres of the teeth, in all the higher animals, afford excellent illustrations here, for they consist entirely of thickened calcified cell walls of peculiar elongated shapes, apposed to one another.

In the mucous membranes, all the superficial cells of their secreting surfaces are of columnar or sub-columnar shapes, and possess nuclei, but every here and there, one of the cells assumes a globular shape, its upper rim bursts, and its contents are partially discharged; by some observers these goblet-cells are looked upon as unicellular glands, with a mucin secreting function, by others they are regarded as the degraded equivalents of the dead scales of the skin noted above, and make room, in this manner, for the younger cells, which are destined to take their places.

Yet a little more deeply seated, within the true glands of the body, the cells are devoid of any distinct envelope, but are possessed of well-developed protoplasmic bodies of a firm consistency, and are nucleated.

Other tissues of the highly organised animal body present cells which are nucleated and possess both protoplasm and cell-walls, yet they lie imbedded in a substance which it is difficult to account for, and which would defy the labours of the histologist, unless he studied the growth of the tissues in question. For instance, the whole group of the connective tissues present this phenomenon—that their cell-walls are being constantly produced from the protoplasm within, which divides, and gives

rise to new cells, which, in their turn, form new cell-walls, until a certain stage is arrived at, when further development ceases. Concurrently with these changes of growth, the outer moieties of the cell-walls become fused together to produce a more or less homogeneous intercellular substance, known as the matrix in hyaline cartilage, whilst the cells themselves retain their individuality intact, that is, they are possessed of protoplasm, nucleus, and cell-wall; the change goes a little further, and the homogeneous-looking matrix becomes fibrillated, in which case the varieties of tissue, known as white fibro and yellow fibro cartilage result, the difference being manifested in a preponderance of the gelatigenous element in the former, and of the elastic element in the latter instance. In every case, however, the cartilage cells remain of an ovoid form; where they subsequently throw out processes, and where the matrix becomes highly calcified and the original cell-wall, too, takes up lime salts, the branches, anastomose, and, finally, the protoplasmic cell processes are withdrawn, the structure known as bone is the result.

To diverge in yet another direction, the cells may throw out filaments which anastomose, and form a reticulated structure; at the same time, they produce a jelly-like material in the interspaces of the reticulum, that is known as mucous tissue, and is the transitory form of embryonal tissue; it obtains permanently in the vitreous humour of the eye. The fœtal mucous tissue develops still further with the growth of the animal, and the mucous intercellular matrix becomes fibrillated; when the gelatigenous element is in excess it is called white fibrous tissue; when the fibres are chiefly composed of the elastic element it is said to be yellow fibrous tissue. Some of the cells of the connective tissue withdraw their filamentous processes or may never develop them; in this condition they possess the power of locomotion, and are termed wandering cells; others remain fixed and immovable, clasping the fibres which they combined to produce, whilst a third set remain fixed, but slowly change their form.

There is yet another interesting type of tissue called retiform or adenoid tissue, which is composed of a dense reticulum organically derived from the coalescence of branched cells. The network thus produced may give rise to cells of other shapes by a process analogous to gemmation, and superadded to all this the interspaces of the reticulum may be filled with corpuscles derived from quite another source, viz., the lymph. Adenoid tissue is characteristic of the spleen and lymph glands in general.

Muscular and nervous tissue again, form a departure from the normal type of the cell in very marked ways. Whilst their cell walls and nuclei remain but slightly altered except in form, their protoplasmic contents undergo profound changes, resulting in the case of striated muscle in a peculiar banded structure, the true nature of which is still involved in considerable obscurity, owing to the complicated questions which have arisen from imperfect illumination and the use of the high powers of the microscope which are required for its elucidation.

Yet all these diverse forms of elements have been called *cells;* from the independent little *Protamœba*, with its apparently structureless jelly-

like body, through the passive calcified cell-wall of the enamel fibres of the teeth, to the complicated cells of striated muscular fibre. No rational attempt to classify them was made until recently, when RUTHERFORD[1] did so in a clear exposition to his students in the University of Edinburgh. The following is a slight modification of his generalisation :—

A cell may be any one of the three following things :—

1. A protoplast, with or without a periplast.
2. An endoplastic or nucleated protoplast, with or without a periplast.
3. A periplast from which both protoplast and endoplast have vanished.

From what has already been stated, it will be gathered that the term cell may be employed to denote a vast variety of forms. It may be pointed out here, however, that even the nomenclature of RUTHERFORD is to a certain extent ambiguous and in more ways than one. For instance, amongst the lowest forms of life there are structures of the simplest description which in certain stages would be classed under the first category, *i.e.*, as simple non-nucleated protoplasts ; but when their life-history is worked out, they can no longer be looked upon as single morphological units, for they are shown to be composed of a number of cells which have coalesced to form a *plasmodium* in which no trace of former aggregation can be detected. The plasmodium of HAECKEL'S *Protomyxa* is of this nature, and amongst certain plants *(Myxomycetes)* the formation of plasmodia is the rule, not the exception. Then again, in speaking of the cells of ordinary connective tissue, only the corpuscular elements are taken into account, although in strict conformity with the definitions given, the fibres of the matrix ought also to be included. So also, in describing the cells of hyaline cartilage, the matrix which they produce should be considered. It would be well if the term *corpuscle* could be retained for such structures as are only the partial derivatives of single cells or morphological units, and the term *cell* confined to the total product of each unity ; in that case the word *blood-corpuscle* would have to be abandoned for *blood-cell.*

The three parts which enter into the structure of a typical cell, are (1) protoplasm, (2) cell-wall, (3) nucleus. The protoplasm is the chief amongst these, inasmuch as without it the cell could not exist ; every cell at some stage or another of its life possessed protoplasm as an integral part, and every living and growing cell possesses protoplasm. The cell-wall may or may not exist, and is, therefore, only of secondary importance, although it may finally come to be the most essential portion of the cell, as in the enamel fibres of the teeth, and in the red blood-cells of the *mammalia.* Unlike the protoplasm, the cell-wall, although endowed with life, is incapable of multiplication ; it cannot of itself, give rise to new cells, whereas the protoplasm can become the seat of cell multiplication. The true nature and functions of the nucleus, with or without its contained vesicle and nucleolus, is not yet understood. Its prevalent division, previous to the division of the protoplasm in cell multiplication, long caused it to be invested with undue importance. The ten-

[1] *A Text Book of Physiology*, Edinburgh, 1880, pp. 27, 29, 30.

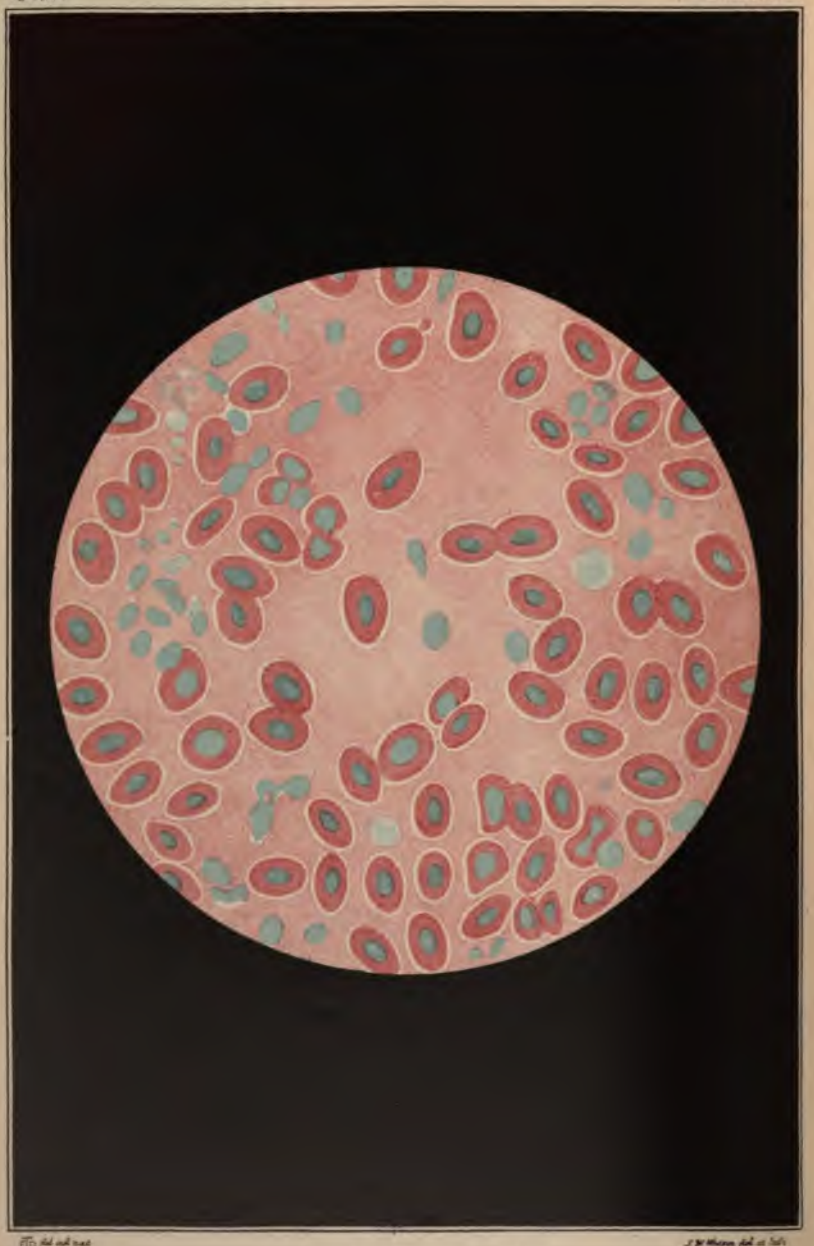
dency now is to regard it as merely a denser portion of the protoplasm, and to doubt its importance as a peculiar structure; until its functions are definitely ascertained, however, it would be presumptuous to attach either an undue value to, or to underrate it. In the young condition of cells which possess nuclei, the latter are of spherical form, and more or less central in position. As the age of the cell advances, the nucleus shows a tendency to assume a parietal position, and to become oval, or even quite flattened. It always exhibits a greater affinity for such re-agents as aniline dyes and carmine than the surrounding protoplasm, and is not so rapidly acted upon by acids and alkalies. On chemical analysis, in certain cells, at all events, *e.g.*, pus, it yields a special albuminoid substance, *nuclein*, which is absent from ordinary protoplasm. The researches of KLEIN, FLEMMING, and others have shown that it possesses a minutely reticular structure, which they term *intranuclear*, in contradistinction to the *intracellular* reticulum, which they have detected in the general protoplasm of the cell. Within the nucleus, one, two, or more nucleoli, may sometimes be observed. These are usually of denser formation than the nucleus itself; their function has hitherto been merely a matter of guess work, and, on account of extreme minuteness, their chemical constitution has not been discovered. Sometimes the nucleus contains a space or vesicle, which is revealed by the double contour of that body under a high power of the microscope.

The reader can readily verify what has been said regarding KLEIN'S demonstrations of an *intranuclear* and an *intracellular* reticulum. Many old horse-ponds contain the common newt or eft, which is easily caught in a small net. Take two newts, confine one in a small quantity of water for a few days (usually about four), and the whole epidermis will be shed as a fine film, giving a complete cast of the entire animal. Place a snip of this in absolute alcohol for twenty-four hours; also, another snip in a saturated aqueous solution of picric acid for the same length of time. A small piece of the former may be stained in logwood solution, and mounted in Farrants' medium, whilst another small piece, hardened in the picric acid—after being washed free from the acid—may be stained in picro-carmine for an hour or more, and also mounted in Farrants' medium. As the film is extremely thin, care must be taken to press the cover-glass down upon the slide, or a flat field will not be obtained. Either preparation will prove a permanent slide, and beautifully demonstrate the intracellular network of fibrils. Cells sometimes have a *granular* appearance, due to the ends of these fibrils being in focus. The other newt must be killed, its mesentery carefully removed, and placed in a five per cent. aqueous solution of chromate of ammonium for twenty-four hours. At the expiration of this time, wash it free of the solution, then snip off a piece, and stain it for several hours in picro-carmine and mount it in Farrants' medium. This also must be well flattened upon the slide in order to obtain a flat field. It exhibits the *intranuclear* fibrillation in the cell of the numerous, half-isolated (in some cases wholly isolated) non-striated muscle fibres. The *protoplasm* of cells has so strong an affinity for the ordinary staining fluids that its bare existence can be easily and con-

stantly detected by the observer when examining parts of plants and animals. It is watery to some extent, but will not mix with water in the living cell. Real granules are frequently imbedded in parts of the hyaline substance of the protoplasm; globules of watery fluid also are sometimes to be observed. The chemistry of protoplasm has not yet been decided, but it is, at least, similar to albumen and usually neutral in re-action, or weakly alkaline. The great elasticity of protoplasm is seen in the cells lining the bladder, the arteries, the air vesicles of the lungs and the pleura. The most interesting feature to the observer, and the most important property of protoplasm, is its curious powers of movement. These are truly wonderful, and are easily observed, but as the movements are slow and uncertain, the observer must exercise patience, and devote, at least, an hour to their study, during which his observation must be continuous. He will also gain a better idea of the attitudes assumed by the protoplasm by sketching, at stated intervals, the form of the cell he is watching, and then comparing the sketches. The best specimens for this purpose are the *Amœbæ*, minute organisms to be found in stagnant water, in mud, or in damp earth. These have the appearance of a particle of jelly. The most readily obtainable masses of living protoplasm, however, if the observer does not object to the prick of a fine needle, are the white corpuscles of the blood. A handkerchief is wrapped tightly round the finger, which is then pricked, and a minute drop of blood placed on a clean slide. The slide is laid on a warm stage—a piece of copper plate, pierced with a hole, will answer the purpose, provided it be long enough to extend beyond the stage, so that a spirit lamp may be used to warm it. The heat must be carefully regulated, because if too great warmth be applied, the movements will cease. Some observers place solid pieces of Cacao-butter on the slide, as beacons for this purpose. Should the white blood-corpuscle be under observation the changes of form will be found to be very similar to those of the amœba, but slightly less active. The first thing to strike the observer is the formation of a *pseudopodium*, which appears as an elevation on some part of the cell, this increases in size, and currents carrying granules flow into it. Sometimes an incoming and an outgoing stream of granules may be seen in one and the same protrusion. It is by means of this throwing out of pseudopodia that the cell is enabled to move from place to place. The process is extruded, the rest of the body follows, and in this manner progression is achieved. *The ingestion of foreign particles* is also a phenomenon easily seen in the protoplasm of amœbæ or the white blood corpuscles. The blood of the newt, by preference, is taken, on account of the larger size and number of the white corpuscles. Take fine granules of carmine, vermillion, indigo, or of aniline blue, which has been precipitated by alcohol from an aqueous solution. These may be mixed with water, or with white of egg, 1 part, and salt solution ($\frac{3}{4}$ %) 2 parts, and strained through muslin. The blood is placed on a slide, and a ring of oil run around the cover to prevent evaporation, and the slide placed upon a warm stage—less warm in case of newt's blood being used. Cohnheim proved the practical importance of feeding the white blood-corpuscles with these pigments, by injecting, for several days in succession, 2 or 3 cubic centimetres of water, containing fine

particles of aniline blue, into a subcutaneous lymph-sac of the frog. The lymph-corpuscles enveloped the blue granules, and entered the circulation. After some days he induced inflammation of the cornea, and found among the pus-cells at the seat of irritation some containing the blue granules, thus proving that they had *emigrated* from the blood-vessels into the corneal tissue. This experiment establishes the assertion that the protoplasm of cells is capable of movement from part to part, and is also capable of enclosing particles with which it comes into contact. The particle will be first observed to adhere to the surface of the protoplasm; then processes from the protoplasm are thrown out around it and by them withdrawn into the interior, where it may remain some time, being carried hither and thither by any currents that may happen to be flowing in any part of the interior, and, of course, carried bodily with the protoplasm wherever that may wander. After a while it may be expelled. Variations of temperature influence the movements of this protoplasm; thus, in warm blooded animals, any cooling of t' e protoplasm below 10° Cent. causes cessation of movement, which, however, is resumed on restoring the warmth; the activity being heigthened up to, and a little beyond, the temperature of the body from which the protoplasm has been drawn, to cease, however, if the abnormal temperature be long continued. *Oxygen*, also, is requisite to keep protoplasm in its active state, though it can go on for a short time without a fresh supply. *Water* appears to act as a stimulant, or rather, perhaps, as an irritant: if absorbed to a slight extent, the protoplasmic movement is accelerated: beyond this, it is destroyed. *Alkalies*, also, when very dilute, act like water upon it, to some extent, but not so acids. The vapours of chloroform and ether rather arrest the movement without destroying its capability, which may be seen to reappear soon after the vapours are withdrawn. Should the student wish to apply these vapours or gases, he can easily do so by placing a drop of the substance on a slide, with a glass cell around it, and then viewing the protoplasm as it adheres to the cover glass, its under surface, of course, being placed over the glass cell.

The preparations of *Globegerina ooze* and of *Polycystina*, have been selected as showing the elaboration of *calcareous* and *siliceous* tests, respectively, by unicellular organisms; as exhibiting the animal cell in its most complex form; and as typical examples of the Foraminiferous and Radiolarian groups. The calcareous shells of *Globegerina bulloides* consist of nearly spherical chambers, progressively increasing in size, and opening into a vestibule common to all. In the living state, floating Globegerinae are met with in the surface waters of the Ocean, and were caught in "tow-nets" by the scientists of the "Challenger" expedition. The shells, during life, will be found to be furnished with numerous delicate calcareous spines, which extend themselves radially from the angles at which the ridges meet to a length of four or five times the diameter of the test. At the bases of these spines the sarcodic substance exudes through the pores of the shell, forming a flocculent fringe around it, which extends itself on each of the spines, creeping up one side to its extremity, and passing down the other with a peculiar flowing movement. The whole of this sarcodic extension is retracted, if a drop of an irritant fluid be added to the wate containing the organisms.



BLOOD OF FROG
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Sir Wyville Thomson maintained that these organisms only live at, or near, the surface, and that, when they die, they lose their spines and sink, thus occasioning a continual rain, so to speak, of Globegerinæ to the ocean floor; whilst Dr. Carpenter asserts that they have been found living at the bottom, having passed part of their lives in the upper waters and sunk when the thickening of their shells rendered them too heavy to float; whilst many types pass their whole lives at great depths. The Foraminifera may be caught, and their habits studied, by carefully examining sea weeds, zoöphytes, etc., to which they adhere. The *pseudopodia* of Polycystina radiate in all directions from the deeper portion of the extra capsular sarcode, and in some species are branchiate and anastomose, whilst in others they are enclosed in hollow rods, which form part of the siliceous skeleton, and issue from the extremities of these. A flow of granules takes place amongst them, and they feed on diatoms and other minute algæ, marine infusoria, etc., in the manner already described.

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THE BLOOD.

Blood of Frog, double stained.

×

Etymology.—Blood *n.* [Anglo-Saxon *blôd*, Gothic *blôth*, Icelandic *blôdh*, German *blut.*] The fluid which circulates through the arteries and veins of men and animals. This is a rough definition. It is more correct to say, that it is the great carrying agent of the body which supplies itself and the remaining parts of the body with air and pabulum, and bears away the used and noxious parts of the various tissues to their respective outlets.

Description.

Blood may be roughly regarded as consisting of a *fluid* (plasma) in which are suspended a number of more *solid* bodies (the red and white corpuscles). If we take hyaline cartilage and blood and compare them we have cartilage corpuscles fixed in a firm "setting." The matrix on the one hand, and blood corpuscles floating in a liquid setting the plasma on the other. Blood is thus seen to be a tissue as cartilage is tissue. It is the great medium of exchange between all parts of the body. Its universal distribution in the body has led Bernard to remark that it is *an internal medium* bearing the same relations to the constituent tissues that the external medium,

the world, does to the whole individual. Just as the whole animal organism lives on the things around it, its air and food, so the several tissues live on the complex fluid by which they are all bathed, and which is to them their immediate food and air. From this it follows that the composition and characters of the blood must be for ever varying in different parts of the body, and at different times.

It is, therefore, with the above understanding that we now proceed to give a physical analysis.

The blood within the arteries is scarlet: within the veins dark purple. It is slightly heavier than water, its sp. gr. being 1·055. It is saltish to the taste, with a slight alkaline reaction, and an odour in all cases peculiar, but in some cases so peculiar that one can at once tell the source from which it comes. The naked eye appearance of homogenity is deceptive, as plainly shown with the microscope whilst in the animal body, by taking a transparent living tissue, such as the web of a frog's foot, frog's mesentery, bat's wing, fish's tail, etc. A drop of blood on a slide, also reveals its complex character, when viewed through the microscope, with even a low power.

The liquor sanguinis or plasma, when the blood is drawn from the body, separates into two parts, a solid and a liquid. The solid gradually becomes solid, and in doing so contracts and squeezes out the liquid. This solid is called *fibrin*, and the liquid it expresses *serum*. In becoming solid the fibrin encloses the corpuscles, and then we have a *clot* with liquid serum on its surface. In other words; if we catch blood in a vessel, such as a basin, as it flows from the body, then let it stand, we find it form two substances, a liquid, the colour of straw; and a solid, almost scarlet. The coloured clot is, as we have said, fibrin, that has entangled red corpuscles and has shrunk. In clotting, the fibrin contracts in all directions, therefore we find its surface saucer-shaped: its sides have shrunk away from the sides of the basin, and the serum it has squeezed out floats both on it and around it, and slightly beneath it. Quain (vol. ii. 9th Ed.) gives a diagram of the above; thus:—

Liquid blood	Corpuscles...		Clot	Coagulated blood.
	Liquor Sanguinis	Fibrin		
		Serum . .		

If we tie a handkerchief tightly round our finger, then prick the end of the finger with a small sharp needle, then place the minute drop of blood which oozes forth on a perfectly clean slide, and cover it, using very gentle pressure to flatten it well out, we find abundant pale-straw coloured corpuscles (the red corpuscles), and here and there a colourless globular body (the white corpuscle). After a while the red corpuscles collect into rolls like a pile of pennies. These red corpuscles are, in man, biscuit shaped: that is, they are round and thicker at the edge than in the centre. Technically speaking, they are biconcave discs. Their average diameter, they vary slightly, is about $\frac{1}{3500}$th of an inch. Should the light pass through several of these, then we see that they are red. These red corpuscles are elastic and very compressible, as may be seen during their circulation. Vierordt calculates that there are 5,000,000 red corpuscles, and 10,000

white corpuscles in a cubic millimeter of healthy human blood. The number of these corpuscles relatively varies much. In fine, healthy, human blood there will be 1 white corpuscle to about 500 red ones. Then, again, those living in towns do well if they can keep the ratio at 1 in 300. Further, in the anæmic the proportion is still lower.

In structure, the red corpuscles are formed of a colour giving crystallizable substance called *hæmoglobin*, and a colourless part called the *stroma*. Water readily dissolves hæmoglobin, so that if a drop of water be added to the drop of blood, the red corpuscles discharge their hæmoglobin and remain spherical colourless bodies. Many other agents also dissolve out the hæmoglobin, dilute acids among these. If a weak solution of tannic acid be used the hæmoglobin is discharged, but being insoluble in the tannic acid it adheres to the surface of the colourless stroma like a dark coloured globule. After its escape from the red corpuscles the hæmoglobin crystallizes into elongated prisms. Rutherford gives the following method for obtaining a permanent preparation of hæmoglobin for the microscope :—"Kill a rat or mouse by the inhalation of ether. Place a drop of its blood upon a slide, and add twice its volume of water. Mix the two with the point of a needle, and allow slight evaporation. Prismatic crystals, either isolated or in rosettes, will be found. These may be preserved by allowing the blood completely to dry before the application of the cover-glass, and then applying a drop of dammar or balsam solution and covering."

The stroma of the red corpuscle, after the hæmoglobin has been washed out, is found to be composed of paraglobulin, cholesterin, and protagon. (Quain).

The blood of the frog which accompanies the present number is seen to differ from the above description mainly in the red corpuscles being nucleated, and the corpuscle being oval, not round. Water causes both nucleus and stroma to swell up, and the coloured part to withdraw. Both coloured matter and nucleus can be expelled from the red corpuscle of the newt or frog by a 2 per cent. solution of boracic acid. One or two nucleoli within the nucleus of the amphibian red corpuscle can be brought into view by dilute alcohol. The nucleus contains a close network giving to it a granular appearance. The nucleus is of the same shape (oval) as the corpuscle itself, and is about one-third the length of the latter. Very likely the term nucleus, and our regarding the nucleus as a separate entity is misleading; because it is not visible in the red corpuscle of the amphibian *within* the body, and in its normal surroundings.

With regard to the existence of an enveloping membrane there are two schools: one which says there is, and adduces as proof, that the action of certain fluids upon the red corpuscle is osmotic, and causes the corpuscle to swell out from the discoid shape to the globular, or to shrink and become crenated, according to the reagent used. The other school refuses to accept this as evidence, and asserts that if the interior of the corpuscle be more fluid than the exterior, the osmotic action would still be present. The latter also point to the fact that no one has ever seen a separate cell wall.

The white blood-corpuscles is not quite so familiar an object, as it requires looking for in examining human blood. Only students see it, on

account of its scarcity, one white corpuscle in hundreds of red corpuscles. In our article on the cell, the properties of these remarkable bodies are dwelt upon at length, the most remarkable among which is the change of form. Ordinarily, however, we may safely regard them as spherical, and any form they assume that is not spherical records a degree of movement, the power being inherent in the corpuscle, and capable of being aroused, as has been already stated, by warmth and other stimuli. They are somewhat larger than the red corpuscle, being about the $\frac{1}{3500}$th part of an inch in diameter. As has been described, it is a minute protoplasmic structure, enclosing one or more nuclei.

Besides red and white corpuscles, there are other bodies discernible, the chief among which are minute round colourless particles, *elementary particles* of Zimmermann or *hæmatoblasts* of Hayem.

Double Staining of Amphibian Blood.

The preparation which accompanies this number has been stained according to the method, slightly modified, of Dr. Allen Y. Moore as described in "The Microscope and its Relations to Medicine and Pharmacy," vol. ii., No. 3, Aug. 1882. Two solutions are used, named respectively A and B.

Solution A.

Eosin.	5 grains.
Distilled water	4 drams.
Alcohol	4 drams.

Dissolve the eosin in the water, then add the alcohol.

Solution B.

Methyl aniline green ...	2 grains.
Distilled water	1 ounce.

Having chloroformed a frog its head is cut off and the blood taken from the parts, or the frog is pithed and blood taken from the heart. Dr. Moore directs the drop of blood to be placed on one slide and the edge of another slide to be drawn over this to spread it out. After spreading it is allowed to dry, then it is flooded with solution A for a few minutes: then the slide is dipped and agitated in clean cold water a few seconds; then it is flooded with solution B a few minutes, and again agitated in water to wash off the surplus stain. It must now be set aside out of the way of dust till quite dry, then balsam or dammar added, and the cover put on.

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EPITHELIUM.

1 Squamous _ Tongue
2 Columnar _ Intestine
3 Ciliated _ Fauces

X 400

EPITHELIAL TISSUE.

× 400 diameters.

Etymology.—From the Greek ἐρι, *upon*, and θελε, *a nipple*.

Most of the free surfaces and membranes of the body are lined by epithelium. It is found on the surface of the skin, also on the inverted skin (mucous membrane) which lines the alimentary canal, lachrymal, nasal, tympanic, respiratory, urinary, and genital passages, and the various glandular recesses and ducts which *open into* these various passages. Then again, under the name of endothelium (from endo, within,) it covers the free surfaces of the serous membranes, which line the *closed* cavities (chest, abdomen, etc.) Lastly, it covers the inner surfaces of the heart, blood, and lymph vessels.

The classification of the various epithelia may either be according to function, or according to shape and arrangement. It will suit the purpose of our present study to use both classifications, taking the latter first.

There are three forms, the first of the three having four varieties. We may tabulate them thus :—

1.—Simple :—
   a. Pavement.
   b. Columnar.
   c. Spheroidal, or Glandular.
   d. Ciliated.
2.—Stratified.
3.—Transitional.

The simple is so called because it is made up of a single layer.

a. Pavement Epithelium.—This is the most extensive variety of the simple. It is composed of flattened cells joined at their edges, or, rather, lying edge to edge like the flags of a causeway. Between each cell is a so-called cement substance, invisible, but which can be stained black with weak solutions of silver nitrate, and thus brought into view. Each cell contains a nucleus, and this, again, contains nucleoli. In order to demonstrate this, scrape the inside of the mouth with the nail or a blunt knife: place the scrapings on a slip, with a covering glass; then irrigate with a weak solution of magenta. To demonstrate the cement sub-

We now draw attention to our other mode of classifying epithelia, viz.: according to function. We may tabulate them thus:—

1.—Protective.
- *a.* Stratified.
- *b.* Transitional.
- *c.* Ciliated.
- *d.* Pavement.

2.—Secretory.
- *a.* Columnar.
- *b* Spheroidal (Glandular)

We thus see that the physiology of epithelium may be regarded under two aspects:—its protective and its secretory. The first, or protective, is mechanical and sensory, thus: the stratified and the transitional protect from friction like a coat of mail, whilst the former protects by virtue of its sensory or tactile properties. Then again the cilia may be regarded as acting mechanically and carrying away noxious particles such as we find entering the air passages. The second function is secretory, which we have as yet to call vital through ignorance of the factors which produce the phenomenon.

Mode of Preparation.

A frog was taken and its small intestines, in pieces, placed in a 5 per cent. solution of ammonium chromate. The head also, with the nostrils slit up, was placed in the same. After forty-eight hours the intestines and head were washed in abundance of water, then placed in picro-carmine for a few hours. The contents of the intestines were scraped out; the nasal septum was scraped, as also the roof of the mouth, and the scrapings mounted in Farrants' medium. We are thus enabled to show on one slide *squamous* epithelium (from the tongue), *columnar* epithelium (from the intestines), and *ciliated* epithelium (from the fauces).

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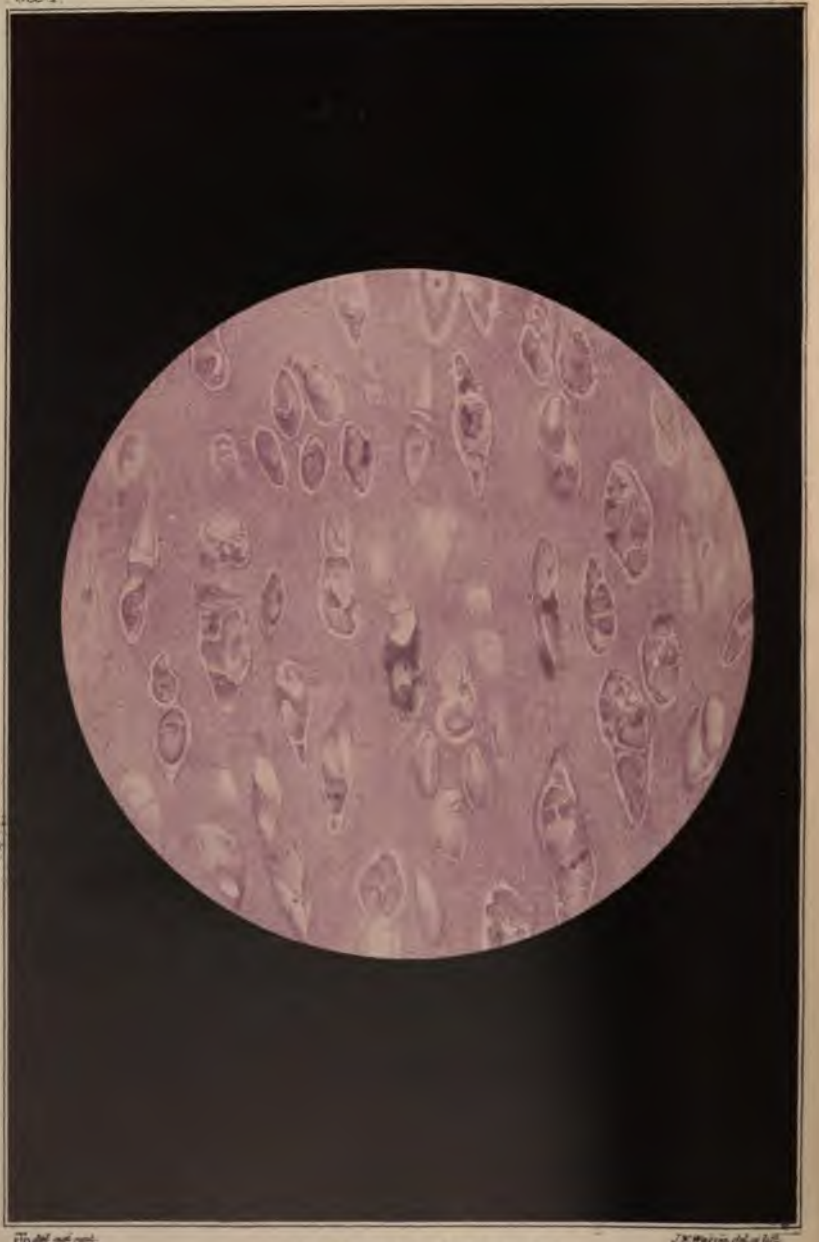

T. S. HYALINE CARTILAGE.
Human Trachea
X 250.

CARTILAGE.

Etymology.—From the Latin, *Cartilago.*

Description.

Cartilage is familiarly spoken of as *gristle*, and forms a most important part of the animal frame. In man and the higher animals, its use lies in its physical property, elasticity; whilst it yields to pressure instantly, it also instantly returns to its own proper form the moment pressure is removed. Its situations in the body alone denote its office; thus we find it as a framework or foundation of tubes which are called upon to bend and twist. At the same time, their passages must be held open, such as the windpipe, larynx, Eustachian tube, and external ear. Again, we find it in the nose and eyelids; whilst the chest or thorax owes much of its elasticity to the cartilages which join the free ends of the ribs to the breast-bone. Its buffer or break-shock qualities are the most strikingly shown by the hard parts of the body, the bones being "shod" with cartilage. Bones are by no means hard, rigid bodies really, but if the long bones, for instance, had not a softer material than themselves to come between at the joints, the act of walking would be impossible from the jar the movement of walking would produce. To meet this difficulty, we find the long bones expanded at their ends, and these ends are shod with cartilage. In situations where jarring is specially to be avoided, we find the approximating surfaces of the joint, themselves shod with cartilage, having a further supply in an interposed cartilaginous pad, as seen in the knee joint, also in the joint of the lower jaw, where masticulating amounting to crunching would jar the brain.

We speak of cartilage as *temporary* or *permanent:* by this we mean that the skeleton of the early embyro, which is nearly all cartilage, but which mostly becomes bone as growth proceeds, is temporary cartilage; whilst that we have described remains as a permanent structure.

Cartilage consists of two parts:

1. Cells.
2. Matrix or intercellular material.

According to the nature of the matrix, cartilage is *classed* as—

1. Hyaline cartilage.
2. Elastic cartilage.
3. Fibro-cartilage.

1. Hyaline cartilage is so called because of its matrix having a ground-glass like appearance (from *L. hyalinus*, glass). We find it at the free end of the ribs: the windpipe is composed of it, and the joint ends of bones have their opposing surfaces shod with it.

2. Elastic cartilage in the adult is hyaline cartilage permeated by elastic fibrils. The fibrils are arranged so as to form the trabeculae* of a reticular† framework; they branch and anastomose very frequently. The meshes contain fusiform groups of large nucleated *cells*, surrounded by a larger or smaller amount of hyaline cartilage substance. (Harris and Power).

3. Fibro-cartilage, or white fibro-cartilage, is found in sessamoid bones, and the approximating parts of the bones of the spinal column, intervertebral substance. Its matrix is *fibrous*, as its name indicates, and is composed of *bundles* of fibres which sometimes form lamellae, with, or without, a concentric arrangement.

Whatever the nature of the matrix, it is permeated by round, or oval, or flattened, *spaces*, called lacunae. These lacunae are formed by a distinct wall, or capsule, cohering intimately with the surrounding matrix, the two being indistinguishable, except we discover the capsule, which absorbs certain stains. The spaces each contain a cell (cartilage cell), or protoplasmic body, generally containing a single nucleus. The cell may entirely fill the space, or it may shrink away from the capsule. These cells multiply by fission, so that we often see a space with two cells in it that have arisen from fission of the one cell which the space previously contained. There are three explanations offered regarding the origin of the capsule as it appears during fission: Kölliker taught that it was an excretion by the cell it afterwards enclosed: Max Schultze regarded it as the converted superficial layer of cell protoplasm; many, however, regard it as a deposit around the cell, with whose origin the cell has had nothing to do. The arrangement of these lacunae, and their contained cells, is to be noticed also their relative sizes. Towards the free surface of a hyaline cartilage the cells and spaces are usually very much smaller than at the parts near the bone which they "cap." Again they may be more flattened, or elongated, and if so, the long axis of the elongation is to be noticed; in the cartilage of the ribs, this axis approaches, in direction, the surface, and may get quite parallel with it.

Cartilage is frequently spoken of as a non-vascular substance but this assertion must be qualified. All cartilages except those of the joints are covered by perichondrium or fibro-vascular membrane. That the constituents of cartilage (cells in a matrix) must come in contact with the blood plasma there is no doubt, therefore whatever the constitution of these channels or spaces may be which this plasma (fluid) travels along they have the carrying or transmitting functions of vessels. The ultimate particles of *any* tissue in exactly the same sense are non-vascular

**Trabs*, a beam. †*Rete*, a net.

and are bathed by the plasma which has been poured forth from the *nearest* vessel so that it is rather a question of *distance* than of vascularity or non-vascularity. The great distance of the cartilage substance from its plasma supply renders its repair when injured exceedingly tardy. A wound or gap in cartilage is first of all filled by connective tissue, which may or may not gradually transform into hyaline cartilage. When cartilage is wounded its margins are much more likely to heal than the deeper parts, in other words, the perichondrium is the main source of the healing factors.

Methods of Preparation.

Cartilage must be prepared in several ways, if we wish to see everything, as no one preparation can show all that is to be seen.

First, we ought to prepare a specimen of each kind of the tissue, by placing pieces, for 48 hours, in a saturated solution of picric acid.

1. Hyaline cartilage may be taken from the trachea, the cartilage of the rib (costal cartilage). or a slice may be taken from the cartilage capping the end of a long bone.

2. Yellow-fibre cartilage may be obtained from the epiglottis of an ox. Place bits in a saturated solution of picric acid for 48 hours, then wash away all the acid, and either cut, stain, and mount at the time, or if not convenient, place it in spirit till required.

3. White-fibro cartilage is best obtained, by cutting away an intervertebral disc, with its adherent bone; placing this in chromic and nitric fluid, until a needle can be thrust easily through the bone; then wash away all the acid, and place in spirit till required.

Hyaline cartilage, stained with logwood, shews the matrix and cells. Take a section and irrigate it with water, which causes the cell to shrink away from its capsule, and to become coarsely granular. Stain sections of costal cartilage, taken from a young kitten, in osmic acid, 1% for 12 hours. This stains the corpuscles a deeper yellow than the matrix. Osmic acid, Iodine solution, silver nitrate solutions, carmine, and eosin are all required in the study of cartilage. The cartilage must also be of fœtal age, adult age, and the cartilage of an aged human being, if possible, ought to be procured, and first, decalcified.

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F.J.O. del. et lith. J. W. Whitson del. et lith.

AREOLAR TISSUE
X 400

Watson & Son Lith. 91 Cr Charles St. Birmingham.

FIBROUS CONNECTIVE TISSUE.

Areolar Tissue × 400 Diam.

Etymology.—Areola (dim. of area, a void space). Areolar tissue is the filmy tissue which largely pervades and connects the different parts of the organs of the body, hence its name *connective tissue*. The older writers called it cellular tissue ignoring its essentially fibrous character. It is often spoken of as the filamentous tissue.

Introduction.

Much confusion exists in speaking and writing of areolar tissue on account of the very numerous terms—many more than we have mentioned—under which it is known. It is a compound of two fibrous tissues (white fibrous and yellow elastic) enclosing cells and spaces (areolae).

Klein, in his brilliant little manual,[1] does much to clear the confusion by dividing connective tissues into "three great groups":—

1. Fibrous connective tissue.
2. Cartilage.
3. Bone.

These he points out have in common a *ground substance* or *matrix* together with, but distinguishable from, *cells*.

In fibrous connective tissue, the *matrix* yields gelatin: in cartilage it yields chondrin; and in bone it is petrified with, and therefore yields, inorganic salts. In fibrous connective tissue the cells are called *connective tissue cells* or *connective tissue corpuscles*: in cartilage the cells are called *cartilage cells*: in bone they are called *bone cells*.

[1] Elements of Histology. Cassell & Co.

White fibrous tissue, its forms and disposal, plus its accompanying cells or corpuscles, form the greater part of the fibrous connective tissues including areolar tissue. White fibrous tissue forms rope like bodies (tendons) which connect muscles with bones. As *fasciae* (from the Latin *fascia*, a bandage) or sheet like expansions, it forms sheaths for the muscles. It forms also a felt work, by its bundles repeatedly crossing and dividing. This felt work may be close and compact, as in the dura mater and sheaths of tendons, or it may be so loose as to allow areas or void spaces (areolæ) as seen under any part of the skin. Lastly, it may form a fenestrated membrane, by its bundles interlacing *in the same plane*. We thus see that its forms and disposal may be regarded as four, namely : 1, Rope-like bodies (tendons) ; 2, Sheet-like bodies (fasciae) ; 3, Open, or close felt-work ; 4, Fenestrae.

White fibrous tissue in our drawing and accompanying slide is seen to be composed of bundles of fine fibres running strictly parallel with each other but taking a wavy course. However the bundles themselves run, whether parallel to each other, as in tendons and fasciae, or dividing and re-uniting and crossing each other as in the felt work, or fenestrated forms, the individual fibres composing the bundles *always* maintain their general parallelism to one another. Other peculiarities of these filaments are that they never divide into branches, or unite with one another. When seen by reflected light they appear white or nearly so ; they, however, readily transmit light and appear transparent. They are to all appearance homogeneous, and the same thickness throughout their course, and vary in diameter from the 50,000 to the 25,000 of an inch. The bundles formed by the filaments vary much in thickness, some being made up of very few filaments, never, however, less then three or four, whilst in others many scores may be seen. The filaments are held together by a semi-fluid albuminous *cement* which is also present between the bundles forming a trabecula. To demonstrate this, tear off a narrow strip of tendo-Achilles from any quadruped: place it in a saturated solution of picric acid for forty-eight hours: wash away all coloring matter by repeatedly changing the water : tear off a small shred and tease it well out in three-quarter per cent. salt solution, then cover and examine.

The *fibrous connective tissue cells* or *corpuscles*, vary much in shape, which largely depends on their surroundings. In tendons and fasciae they are flattened squares, or oblongs, placed end to end, situated on the surface of groups of fibre bundles. Between the ends of the cells is found the *albuminous cement*. In mucous membranes, true skin, serous membranes, cornea, and subcutaneous tissue, the cells are flattened and branched, branches of one cell joining with those of another, thus forming intercellular communication.

Yellow elastic tissue is usually associated with the white fibrous tissue in most places. When seen in bulk as in the ligamentum nucha of large quadrupeds, such as the horse, ox, &c., it is seen to be yellow in colour, but when single fibres are seen by transmitted light, as in the slide

accompanying this number, they appear transparent with a well-defined outline. We can always readily distinguish them from the white fibrous tissue, which we have seen runs in wavy parallel bundles of filaments; the fibres of yellow elastic tissue run in bold curves, and the *ends* of the fibres *curl*. Again, we saw that the white filaments never branched, the yellow fibres branch, and not only so, but the branches join and form a reticulum. When cut across, the yellow fibres are seen to be *angular*. When much white fibre is present, and very little yellow elastic, the latter is discovered by irrigating the specimen with a one per cent. solution of acetic acid in water. This causes the white fibrous tissue to swell up and become indistinct and leaves the yellow fibres alone and untouched; it also, by the way, brings the cells into view.

AREOLAR TISSUE.

By our foregoing remarks it will be seen that the practice of describing areolar tissue as a special variety of a fibrous connective tissue is misleading. It is really a typical connective fibrous tissue, made up of larger or smaller bundles of white fibrous tissue copiously mixed with yellow elastic fibres, the two blending with one another to form a felt work, usually of loose texture. The yellow fibres run in the *ground substance*, between the white bundles, and appear very often, as in our specimen, to run on the surface. The areolæ or interspaces are *deep*, and freely communicate, being formed by the liquefaction of the ground substance and development of fibres, and are occupied by the connective tissue cells or corpuscles.

Except in the eyelids and one other part, the areolar tissue, which lies beneath the skin in all parts of the body, and is therefore called subcutaneous tissue, contains fat cells. Each cell is formed of a capsule of protoplasm, having at one part of its periphery a more or less flattened nucleus, and encloses a globule of oil. These fat cells are formed by the conversion of the original connective tissue corpuscle. When men and animals "grow fat," it is this conversion of the subcutaneous connective tissue corpuscles into fat cells which gives the body its rotundity. In starving animals the oil gets absorbed, and the fat cell diminishes, and contains only a serous fluid, which itself disappears as starvation proceeds. It is thus seen that the areolar tissue beneath the skin is the great store house of the body. The fat is stored up in it in good times, to be called upon by the body's needs in time of want.

Areolar tissue next after the blood is the most universally distributed of the tissues. As we have said, it lies beneath the skin everywhere, also beneath the serous and mucous membranes, binding them to the parts which they cover or invest. It pervades and also invests the different organs, such as muscles, nerves, blood-vessels, bone, and lobules of compound glands, the hollow viscera, such as the stomach, etc. It is the

only universally distributed, but is continuous with itself throughout. It is through the areolæ of this tissue that our medicines, injected beneath the skin anywhere, get into the blood instantly and over the whole system; it is in this that dropsy lodges, and freely shifts its quarters from ankles to eyelids and face, or any other part, often depending upon position only. In the days before the legal protection of animals from cruelty, the butcher whitened his veal by bleeding the calf almost to faintness a day or two before killing it, and after piercing the skin, inserted his tobacco pipe stem, and inflated the entire skin by blowing into the areolar tissue. Should a quadruped have an abscess form and discharge, or receive a cut through the skin over the breast between the fore legs, the action of the large pectoral muscles suffices to suck in air, and the entire skin is puffed up by the air being drawn into the areolæ of the subcutaneous tissue.

It is thus seen that the areolar tissue is our store-house of fat: it receives watery deposits from the blood-vessels: it acts as a convenient receptacle for medicaments, whether injected beneath the skin with a syringe, or rubbed into the skin by inunction: it enables the skin to move freely over the subjacent parts, and retracts the skin when the latter is drawn away, and makes the cut skin gape by drawing its cut edges apart, for which we do not thank it. Its other kindly (and unkindly) offices, are really "too numerous to mention."

Mode of Preparation.

For studying areolar tissue, snip out a minute piece of the subcutaneous tissue from a newly-killed quadruped: place it quickly on a dry slip, and spread it out with needles, breathing upon it from time to time to prevent it drying, then place on it normal salt solution, cover, and examine.

For a permanent specimen, inject the axilla of a newly-killed kitten or young rat, with a 1% solution of osmic acid. When a bulla has formed, snip out the areolar tissue, avoiding hairs, place it on a slip as before, and put on it a large drop of picro carmine, frequently renewed through 24 hours; then replace this with glycerine jelly and cover. Logwood may be used in place of picro carmine; also glycerine may be passed under the cover glass, instead of using the glycerine jelly.

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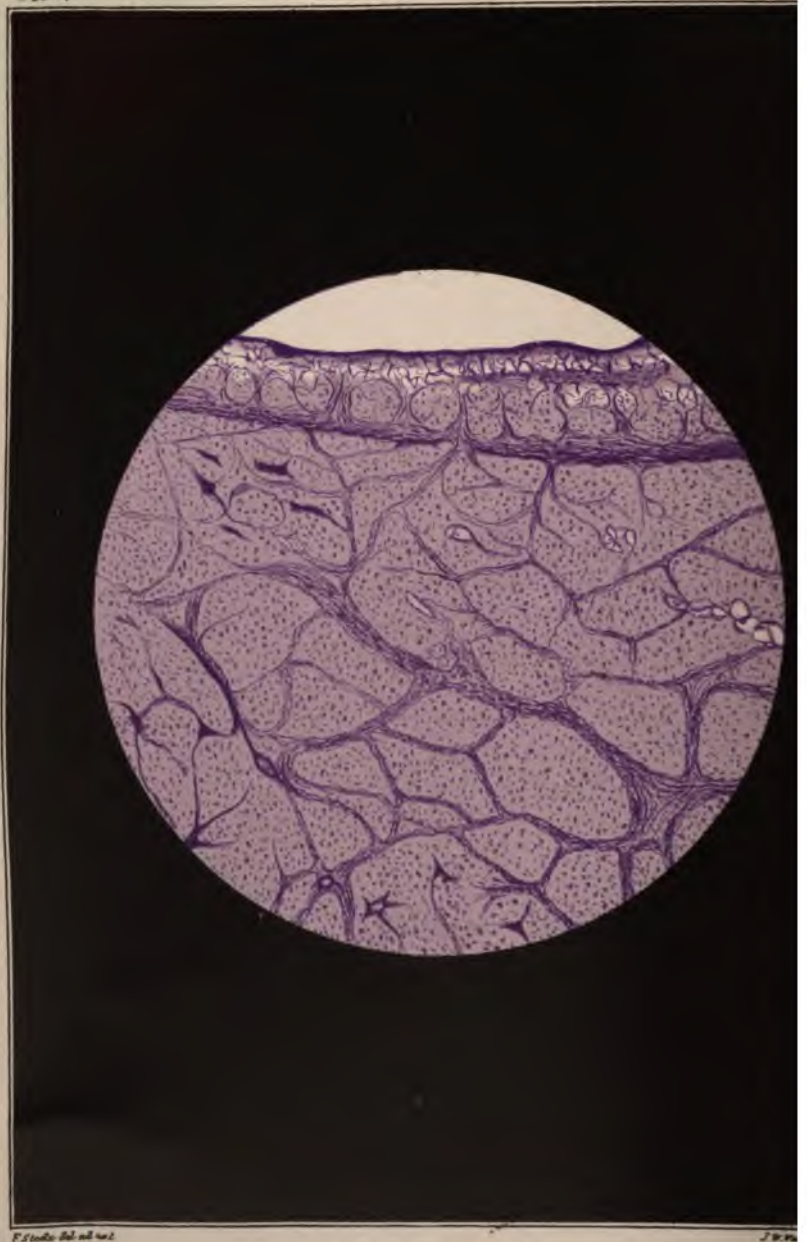


TENDON OF LAMB

X 70

FIBROUS CONNECTIVE TISSUE.

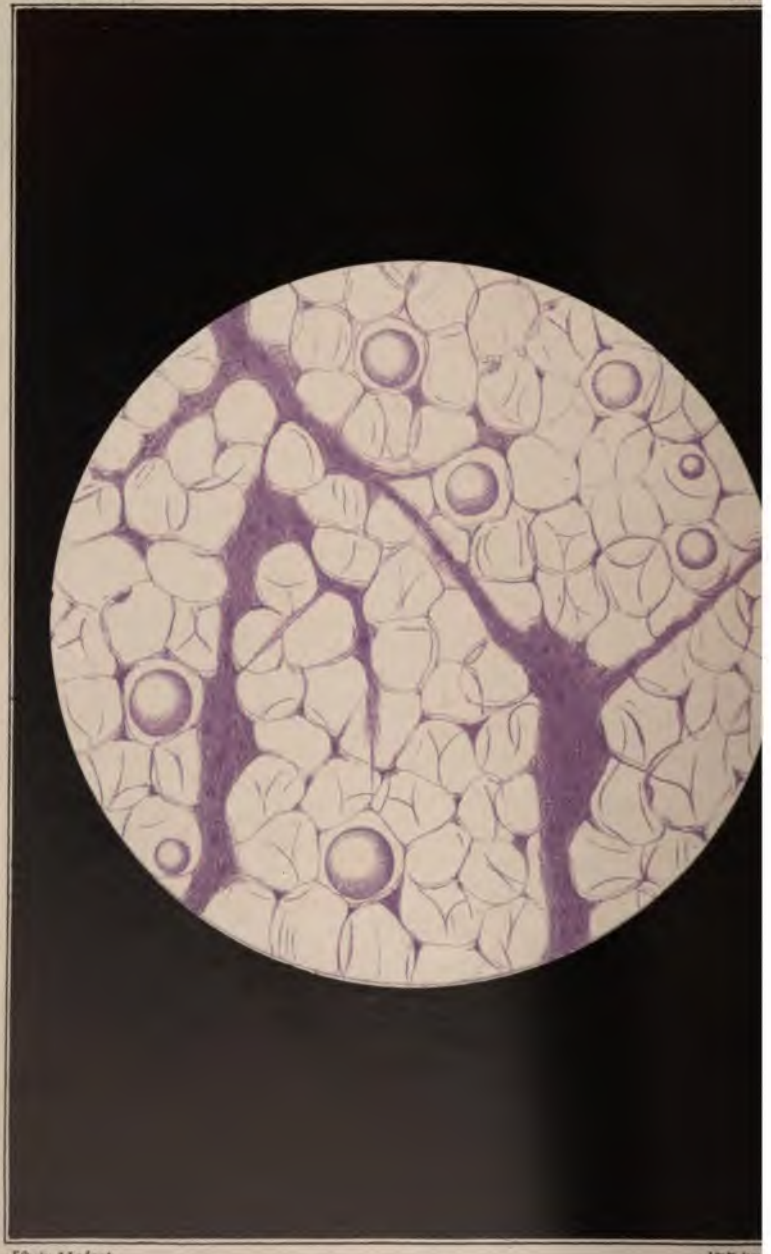
(Continued.)

Tendon T.S. × diam.

Etymology.—(τένων, a tendon, from τείνω, I stretch). A fibrous cord at the extremity of a muscle by which a muscle is connected with a bone. Tendons are white and glistening, and vary in length and thickness: they are sometimes round, sometimes flattened: they are of considerable strength, with very little elasticity.

Description.

Tendons consist almost entirely of white fibrous tissue, the fibrils of which have an undulating course parallel with each other, and are firmly united together. The bundles of fibres do not keep separate throughout their length, but send slips *to* neighbouring bundles, and receive slips *from* neighbouring bundles in return; hence, successive transverse sections of the same tendon reveal different figures of the sectional areas of the bundles. On referring to the present slide, it will be observed that the bundles are not only invested by dense areolar tissue, but trabeculae are sent from this into the interior, forming septae of greater or less thickness, thus uniting all the bundles of fibres into one large collection of fibres. Besides binding the bundles together, these septae of areolar tissue serve as an imbedding and protecting agent for the vessels and nerves, which, however, are very sparingly supplied, and of small size, running for the most part in the larger trabeculae and sending communicating branches across them, and then forming an open network with large oblong meshes. If tendons seem to lack blood vessels, they at all events are richly supplied with lymphatic vessels which penetrate both the large and small trabeculae of the areolar tissue. The present slide exhibits these in numerous places transmitting light from the mirror as if they were so many pin holes. Their areolar character of is shown by their irregular outline, also by the fibres running across each at a lower level. The tendon cells are seen in section as irregularly stellate. They are stained, and give each bundle of fibres a dotted appearance, as seen with a low power such as an inch. The tendon cell consists of a protoplasmic body, thick at the centre, and thinning off into extensions, hence its stellate form. When seen in a longitudinal section of a tendon they *appear* quadrangular or oblong. As the body of the cell lies between the angular space between three or more bundles of fibres its lamellar processes extend into the interstices between these contiguous bundles. It will thus readily be seen that the oblong form of the cells as viewed from the side is quite consistent with their



T Steele del ad nat. J W Watson

ADIPOSE TISSUE

X 250

Watson & Son lith. 31 St Charles St Birm.

ADIPOSE TISSUE.

× 250 diameters.

Etymology.—The word adipose is from the latin *adeps*, *adipis*, fat.

FATS.

These substances, like the sugars, are derived from both animal and vegetable sources. There are three principal varieties of them, which may be considered as representing the class, viz. :

Oleine - -	C_{94}	H_{87}	O_1
Margarine - -	C_{76}	H_{75}	O_{12}
Stearine - -	C_{142}	H_{141}	O_{17}

The principal difference between the oleaginous and saccharine substances, so far as regards their ultimate composition, is that in the sugars the oxygen and hydrogen always exist together in the proportion to form water; while in the fats the proportion of carbon and hydrogen are nearly the same, but that of oxygen is considerably less. The fats are all fluid at a high temperature, but assume the solid form on cooling. Their melting points vary very much, and are :—

Stearine,	143° Fahr.	
Margarine,	118°	„
Oleine,	100°	„ and less.

The fats are all insoluble in water, but readily soluble in ether. By prolonged boiling in water with a caustic alkali, they are decomposed, and as the result of the decomposition there are formed two new bodies; first, *Glycerine*, which is a colourless, neutral fluid : and, secondly, a fatty acid either oleic, margaric, or stearic, as the case may be. The glycerine remains in *a free state*, whilst the acid *unites* with the alkali to form a *soap*.

Stearine is best obtained from beef or mutton suet. It is the hardest of the fats, and crystallises in white shining plates.

Margarine (*μαργαρίς*, a pearl), or mother-of-pearl fat, is a constituent of all oils, hardening rapidly and assuming a crystalline form which glitters like mother-of-pearl.

Oleine may be obtained best from olive oil. Stearine, margarine, and oleine never occur separately: in every fatty substance they are mingled together, so that the more fluid of them hold in solution the more solid. Generally speaking, in the living body these mixtures are nearly fluid. After death, as the body cools, the stearine and margarine sometimes separate from the mixture in a crystalline form, since the oleine can no longer hold in solution so large a quantity of them as it was capable of doing during a life temperature.

In a fluid state fatty substances present themselves under the form of drops or globules, which vary greatly in size but always assume the same optical properties. They are circular in shape and have a faint amber colour, distinct in the larger globules, less so in the smaller. They have a sharp, well defined outline, and refract light strongly, and act like double convex lenses, and therefore appear with bright centres surrounded by darker borders.

Pereira gives the following per centage of oily matter in some animal and vegetable foods:—

Quantity of fat in 100 parts.

Filberts.....	60·00	Ordinary meat...	14·30
Walnuts	50·00	Liver of ox	3·89
Cocoa-nuts	47·00	Cow's milk	3·13
Olives	32·00	Human milk.....	3·55
Linseed.....	22 00	Ass's milk	0·11
Indian Corn.....	9·00	Goat's milk	3·32
Yolk of Eggs ...	28·00		

Fats or oils can easily be extracted from organised tissues by the employment of simple *mechanical* means, because the three principal varieties of oleaginous matter, though always united with each other, *are not united with the other proximate principles.* Thus, suppose we wish to extract the *sugars* of the blood, we should find them in solution in water in company with albumen, phosphate of lime, chloride of sodium, and the like in *molecular union.* On the other hand, we may remove the yolk of an egg *en masse*, churn the fat from the milks, cut animal or vegetable tissue into small pieces, and subject it to heat and pressure, and the oil is forced out and separated. The oils are found in the animal body *most abundantly* in the adipose tissue though they exist in smaller amount in the mammary gland as milk, in chyle, in the liver cells, etc. A large part of the fat, in whatever form, may be accounted for by that taken with the food, animal or vegetable, but it would seem, from the experiments of MM. Dumas and Milne-Edwards on bees,[1] M. Persoz on geese,[2] and M. Boussingault on geese, ducks, and pigs,[3] that fat is *formed* in the

[1] Annales de Chim. et de phys., 3d Series, vol. xiv., p. 400.
[2] Ibid., p. 408.
[3] Chim. Agricole, Paris, 1854.

body independently of what is introduced with the food. The observers first ascertained the quantity of fat existing in the whole body at the commencement of the experiment. The animals were then subjected to a definite nutritious regimen, in which the quantity of fatty matter was duly ascertained by analysis. The experiments lasted variously from thirty-one days to eight months, then the animals were killed and examined. The results showed that considerably more fat had accumulated in the tissues than could be accounted for by the fat of the food taken, which placed beyond doubt that the system can form fat by the metamorphosis of other proximate principles.

Although the food taken as fat, may produce most fat, other substances produce fat in abundance. In sugar-growing countries, such as Louisiana and the West Indies, during the few weeks occupied in gathering the cane and extracting the sugar, all the negroes employed on the plantation, and even the horses and cattle, that are allowed to feed freely on the saccharine juices, grow fat, and lose their superabundance of fat when the season is past.

ADIPOSE TISSUE.

As we have said, by far the greater part of the fat of the body, is enclosed in small cells or vesicles, which, together with their contained matter, constitute adipose tissue.

When seen under the microscope, the tissue is found to consist of small vesicles filled with oily matter and mostly lodged in the meshes of areolar tissue. "The vesicles are most commonly collected into little lobular clusters, and these again into the little lumps of fat which we see with the naked eye, and which in some parts are aggregated into round or irregular masses of considerable magnitude. Sometimes the vesicles, though grouped together, have less of a clustered arrangement; as when they collect alongside of the minute blood-vessels of thin membranous parts. In well nourished bodies the vesicles or fat cells are round or oval unless they are packed closely together, in which case they acquire an angular figure, and bear a striking resemblance to the cells of vegetable tissues. The greater number of them are from the $\frac{1}{200}$ to the $\frac{1}{100}$ of an inch in diamater, but may exceed or fall short of this measurement. Each one consists of a very delicate envelope enclosing the oily matter, which, completely filling the envelope, appears as a single drop. It often happens that a part of the fatty contents solidifies in the cell after death, forming a bunch of delicate needle-shaped crystals. The envelope is the remains of the original protoplasm of the cell: it is generally quite transparent, and apparently homogeneous. According to some authorities it consists of two parts: a delicate structureless external membrane, and a layer of finely granular protoplasm immediately surrounding the fat. The

nucleus is always present in the protoplasm, but is often so flattened out by the pressure of the enclosed oil-drop as to be visible only with difficulty. The areolar tissue connects and surrounds the larger lumps of fat, but forms no special envelope to the smaller clusters; and although fine fasciculi and filaments of that tissue pass irregularly over and through the clusters, yet it is probable that the vesicles are held together in these groups mainly by the fine network of capillary vessels distributed to them. In the marrow the connective tissue fibrils are but few in number or may, it is said, be absent altogether. The adipose tissue is copiously supplied with blood-vessels. The larger branches of these pass into the fat lumps, where they run between the lobules and subdivide till at length a little artery and vein are sent to each lobule, dividing into a network of capillaries, which passes between the vesicles in all directions, supporting and connecting them. The lymphatics of the fat are in close relation to the blood-vessels, accompanying and occasionally completely enclosing them as they enter the lobule. No nerves have been seen to *terminate* in this tissue, although nerves, destined to other tissues, pass through it." (QUAIN.)

METHOD OF PREPARATION.

Our section with this number is from the subcutaneous tissue of the palm of the hand. It is cut by the imbedding process, and stained with strong logwood solution and mounted in glycerine jelly.

BIBLIOGRAPHY.

All works on anatomy may be consulted, as all treat on adipose tissue with greater or less lucidity. In the 9th edition of QUAIN vol. ii. p. 73, 74, 75, will be found a good description, from which we have made a large extract. Works on Physiology also have a good deal to say about the fats, etc., so have works on Chemistry.

J. W. Watson del. et lith.

OSSIFICATION OF CARTILAGE

(Quain.)

X 300

Watson & Son. Lith. 91 C.t Charles St. Birm.

DEVELOPMENT OF BONE.

Section × 350 diam.

Etymology.—Several terms used in discussing bone formation require to be explained or known in advance. The following words occur: the prefix oste from the Greek *οστεον* (a bone). Osteoblast from the Greek *βλαστος* (a bud, a shoot, a sucker). Osteogenetic from the Greek *γενναω* (to produce, to bring forth). The prefix os from the Latin os (a bone). The prefix calc from the Latin calx (lime). Diaphysis and Epiphysis are the same word with different prefixes. They are from the Greek *φυω* to spring forth or come into being hence *διαφυω* to intervene: *επιφυω* to grow upon, to cling closely to.

Introduction.

In order to understand the development of bone, we first propose to explain what bone is when fully formed, as we find it in the full-grown healthy animal; then we have to begin quite at the other end and trace the process from the primitive structures as we find them, not only in the fœtus but in the young or very early fœtus; thus, we count an animal's age from its birth, but before birth we count its age as a fœtus. We again have to allow for differences in fœtal development; thus a fœtus which is mature and ready to be born, so to speak, at six weeks old is mature and formed at that time and almost so at four weeks, whereas a fœtus that is only fit for a separate existence at twelve months is very imperfectly formed at four weeks. Therefore when we speak of an early fœtus or young fœtus, we must be understood to mean an early or young fœtus of the animal which bears it, and then consider how long that animal carries its young before birth.

When bone is fully formed it consists of an organic framework impregnated with lime and other earths, which make it rigid.

Lehman gives the following proportions:—

Organic matter	...	...	...	33 per cent.
Earthy matter	...	...	...	67 „

The earthy matter is almost all lime and is made up of 57 parts of the phosphate and 8 parts of the carbonate of lime: the only two remaining earthy parts are one part each of fluoride of calcium and phosphate of magnesia. Practically, then, we find bone to be made of an organic base, impregnated with lime, which has been named "bone earth." If

The process of ossification commences, as we have said, in the centre of the ossifying part, and proceeds *from* the centre, in the case of the long bones, from the centre to the extremities, and in the irregular bones, or the irregular parts or epiphyses of a long bone, from the centre to the periphery. Hence in our present section we see ossification at work in two parts, first it is seen in the piece of the end of the shaft proceeding in a looped manner, the loops lying lengthwise in the long axis of the piece of the end of the shaft; second, it is seen in the centre of the half sphere or head of the bone. In both cases the fragile open network of new bone will in places have dropped out in process of cutting and mounting. The osteoblasts are stained red, and amongst these cells may be found here and there giant cells or osteoclasts. The trabeculæ are stained blue, and are calcified cartilage matrix in process of becoming covered with secondary osseous substance deposited by the osteoblasts. Being taken from an animal that uses its limbs early, the process of ossification in the shaft and head of the bone is going on synchronously. "The young lamb or foal can stand on its four legs as soon as it is born; it lifts its body well above the ground, and quickly begins to run and bound. The shock to the limbs themselves is broken and diminished at this tender age by the division of the supporting long bones, by the interposition of the cushions of cartilage between the diaphyses and the epiphyses." (Owen).

2. *Ossification of Membrane.*—For observing this a very young fœtus must be procured. Quain's Anatomy gives a figure (p. 102, fig. 100, vol. ii., Ed. ix.) of Sharpey's. It is the parietal bone of a fœtal sheep, the latter being only two and a half inches in length. The bone is about half an inch square. Should the reader desire to study ossification in membrane, a very young fœtus of a rabbit, cat, dog, or guinea pig, may be procured, stained first in carmine, then in sulph-indigotate of soda, and then cleared and mounted in balsam. He will then find a brief but clear description as above. It is obvious that a description here would be of little use without a slide or a drawing.

Mode of Preparation.

A new born puppy, kitten, guinea-pig, or rabbit is to be procured and its thigh or arm bones, or both, placed in a saturated watery solution of picric acid till a needle can be run through the bone easily and meet no grit. Then the acid is washed out with water and the bones placed in methylated spirit for a few days. Sections are then cut with a razor, the bone being imbedded in carrot. The sections are first stained in carmine, then in sulph-indigotate of soda (the process having been described in the Studies once or twice before, we need not trouble the reader with a repetition), and afterwards mounted in benzole balsam.

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Any work on Anatomy may be chosen, but the second volume of the ninth edition of Quain (Longmans & Co.) is the best.

E.D. del. et lith. J.W. Watson. del. et lith.

T. S. NERVE OF HORSE.

X 150

Watson & Son. Lith. 93 Ct. Charles St. Birm.m

NERVE OF HORSE.

T.S. [illegible]

Etymology.—*Afferent* [illegible] *ef*, **from.** *Neuroglia*, from the Greek [illegible] **glue, or** *gloios*, anything sticky. *E*[illegible] *neurium* **from** *peri*, around [illegible] **Latin** *intus*. *Neurilemma* [illegible] **peeled off.** All the latter are from the Greek.

The Nervous System.

Before describing any part of the nervous system it may be well to briefly describe the nervous system as a whole.

The nervous system, for descriptive purposes may be divided and composed of three parts, namely:—

Terminals, which receive impressions.
Transmitters, which convey these impressions.
Interpreters, which analyse and interpret these impressions.

It will now be convenient to take these in the reverse order.

The *brain* and *spinal cord* are the great interpreting mechanism; besides these there are numerous little minor centres which we may leave out of consideration in our description.

The *nerves* are the conveyers of impressions from the terminal receivers to the brain and spinal cord, and other nerves which connect the two extremities.

The terminals vary greatly in form but are really the same kind of thing. The agent [illegible] impressions received, and make an impression [illegible] ference differs in each case. Take for example [illegible] their terminals: we have refracting media [illegible] and rendering these beams suitable [illegible] transmitted by the optic nerve; we have [illegible] modifying sound waves before they [illegible] along the auditory nerves, and so on [illegible] optical apparatus, and in the latter [illegible] *instruments* of the terminals [illegible]

If we disconnect the nerves from the central nervous system proper, we find it to be composed of two structures, namely:

1. Nerve cells.
2. Nerve fibres.

The nerve cells are [illegible] every nerve fibre [illegible]

nerve cells are made up of a body which branches. Sometimes these branches are very numerous, and each branch is continued as a nerve fibre, or has a nerve fibre running into it. We have said the fibres commence, or terminate, in nerve cells. This leads us to remark that nerve fibres convey impressions to and from the nerve centres.

Those fibres which convey impressions *from* the end organs, or terminals, *to* the centres (brain and spinal cord) are called *afferent* or *centripetal:* those which carry the brain or spinal cord explosions or stimuli *to* the end organs, or terminals, are termed *efferent* or *centrifugal.*

By the unaided eye we can see that the nervous substance is divided into two kinds, differing in colour. These are :—

1. The white.
2. The grey, or cineritious.

The white part of the brain and spinal cord is very distinct from the grey, and is made up of nerve fibres. The grey or cineritious matter is formed largely of nerve cells. It exists on the surface of the brain and in the interior of the spinal cord.

Nerve Fibres.

We must be careful to remember the difference between nerves and nerve fibres. When we speak of a nerve, we refer to a number of nerve fibres, which run in company as a cord or nerve. It is usual to speak of two kinds of nerve fibres, the white and the grey ; but a white fibre is made up of a central axis of grey matter, which runs uninterruptedly from a cell process to its destination, and is surrounded, or coated over, with a thick white fatty coat called the *medullary sheath*, and this, again, is enclosed in a thin membrane or sheath. In other words, a *white* nerve fibre is made up of three distinct parts :—1, a central axis of grey matter ; 2, a thick white fatty sheath ; and 3, an envelope, or thin membrane, which encloses the whole. On the other hand, a *grey* nerve fibre has the central axis of grey matter almost exactly like that of the white nerve fibre, but running along, just as grey matter, and not surrounded, or coated, by anything.

Nerve Cells.

A nerve cell, like any other cell, consists of a nucleated mass of protoplasm having from one to twenty or more processes : hence we speak of uni-, bi-, or multi-polar nerve cells. The *shape* of the cell is characteristic of its locality. The round cell belongs to the spinal ganglia: the angular to the sympathetic ganglia : the irregular branching cells to the grey matter of the spinal cord : the conical to the surface of the brain : the flask or retort shape to the cerebellum, etc. The *size* of the nerve cell varies greatly. The multi-polar nerve cells in the spinal cord are so large that one can almost see them with the unaided eye, whilst those in a part of the cerebellum are so small that the layer they form is called the granular layer.

Like all other parts of the animal economy, the essential parts of the nervous system (nerve cells and fibres) require mechanical support:

require blood vessels, nerves, and lymphatics for their nourishment and continued existence during their wear and tear.

Without physical support, the delicate grey matter would be crushed or torn, especially in the large collections of nervous substance (brain and spinal cord). The physical support of the nerve cells and fibres of the brain and cord is called *neuroglia*. It consists of an extremely fine, delicate, open net-work, whose structure has not been thoroughly agreed upon. Professor Schäfer, in the second volume of Quain's anatomy, p. 149, says:— "It is not composed of cells, although these occur in it, but it is rather of the nature of an intercellular substance which occupies the interstices between the nerve fibres. The cells which are here and there found in it are flattened, and resemble small connective tissue corpuscles, and the neuroglia of the nerve centres has generally been regarded as consisting of connective tissue ground substance, especially since in many places it appears fibrillated."

Transverse Section of a Nerve.

The transverse section of a nerve, such as accompanies the present description, shows the following structure.

We have one, two, or more nerve *bundles* of different sizes, mechanically supported and held together by a fibrous arrangement of areolar tissue. The latter tissue also affords mechanical support to the nerves, arteries, veins, and lymphatics which must accompany the nerve trunk as a whole, and afford it proper nourishment.

The sheath which surrounds all the round bundles of fibres is that which we first come across in dissecting a nerve as it lies in the body, and is called epineurium. Besides this each nerve bundle has a special sheath for itself, also readily seen: this is called the neurilemma, and is *lamellar* in structure, whilst the epineurium *interlaces with itself* in all directions. The neurilemma *receives* fibres on its outer periphery from the surrounding epineurium: it also *gives off* flattened prolongations from its internal periphery, which go to form septa between the *groups* of nerve fibres which compose a single nerve bundle. From these septa again there is still a smaller structure given off, which separates the individual fibres of which a group is composed. This is called the endoneurium. This endoneurium also supports the capillary blood vessels which nourish the nerve fibres.

Modes of Preparation.

To prepare a Nerve.—Dissect out a piece of the sciatic nerve of a horse, ox, dog, or cat. The piece should be an inch long and gently stretched upon a piece of wood, or a match, and tied at both ends to prevent shrinking if taken from a small animal. If a large animal be chosen the nerve trunk must be cut lower down where it is smaller. Place the cut piece for ten days in Müller's Fluid. Then transfer to alcohol as usual. Transverse sections, stained, then cleared and mounted in balsam, shew the parts we have mentioned in their relative order.

Sections cut longitudinally should also be made. Although the nerve fibres themselves do not coalesce, the minute bundles which go to form a nerve bundle both give and receive fibres from neighbouring bundles.

To prepare a Nerve Bundle.—Take a mouse just killed: make an incision through the skin in the middle line along the breast bone, and tear away the skin right and left. Very fine lateral branches will be seen stretching from the flesh of the chest to the skin, more or less surrounded by areolar tissue. Pour over the entire parts so exposed a half per cent. solution of nitrate of silver: then after five minutes cut out little lengths of these minute nerve trunks and wash them in two or three lots of distilled water in a watch glass. After the light has changed them to a faint grey colour, mount them in glycerine jelly. We then can see the nerve fibres in the form of a bundle, and this bundle coated by a single layer of epithelium. The intercellular substance of the epithelia is darkened by the silver, and we have silver lines well defined. Here and there we see beneath this epithelial mail little crosses which we shall describe lower down.

To prepare Single Nerve Fibres.—We have seen that the medullated nerve fibres have a coat of fat over the central grey band or axis. As osmic acid blackens fat we use it to distinguish this thickest, largest part of a nerve fibre. Kill a frog and dissect out a piece of the sciatic nerve an inch long. Tie it lightly but firmly to a match with a piece of fine thread, wetting it with distilled water all the time to prevent it drying. After cutting it away place it, match and all, in a 1 per cent. solution of osmic acid for ten minutes. Remove it and wash away all the osmic acid with distilled water, then place the whole in a test tube and cover it with a solution of picro-carmine, and gently secure the end of the tube with a cork. Let it remain a fortnight (Stirling), and cut away lengths of one-eighth of an inch, and dissociate the fibres with needles by means of a dissecting microscope. After separating or isolating a few individual fibres, mount these in glycerine jelly. We now see the three components of the nerve —the axis cylinder in the centre, the large fatty (medullary) sheath stained black with the osmic acid, and the whole enveloped in a sheath. We now notice that the axis cylinder is an unbroken, continuous strand; so is the outermost sheath, but this sheath forms constrictions now and again, and in doing so constricts everything except the central axis; therefore, the fatty coat is squeezed away, so to speak, leaving the outer sheath and the central axis only to represent the nerve fibre. It is this constricted bit of sheath running *transversely*, and the central axis here seen, running, of course, *longitudinally*, which stain in the silver preparation of the mouse's nerve, and thus produce the little black crosses here and there along the course of a medullated nerve fibre.

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HUMAN CEREBELLUM.

T S X 150.

HUMAN [illegible]

[illegible]

Etymology.—*Cerebrum* [illegible] brain, [illegible]. Encephalon [illegible] or sickle. Fossa (from [illegible] layer of a flat form. [illegible] itself. Pedicles (from [illegible] investing the whole surface [illegible] (pinnatus festioned). [illegible]

[illegible]

The great accumulation [illegible] consists of four [illegible]

[illegible]

By far the largest [illegible] the two hemispheres [illegible] the head are [illegible] a section [illegible] have only a [illegible]

The cerebrum [illegible] Immediately [illegible] the pedicles [illegible] pedicles [illegible] medulla oblongata [illegible] like the [illegible] itself.

The pons Varolii, or tuber annulare, is a thick band of nervous matter arching forward, and stretching, as we have said, between the two halves of the cerebellum. It is made up of transverse (commissural) fibres, which are pierced by longitudinal fibres passing up from the spinal cord to the cerebrum.

The medulla oblongata appears to be the uppermost part of the spinal cord, but lies within the cranium, hence it is said to be a part of the encephalon. It is wedged between the pons Varolii and the cerebellum: the former spans it and partly encloses it. It is irregularly cone shaped: the thicker part of the cone being uppermost.

"The cerebellum or hinder brain consists of two lateral hemispheres joined together by a median portion called the *worm* or *vermiform process*. This is seen on the under surface in the fossa between the hemispheres, as a well-marked projection named the inferior vermiform process, but above forms only a slight elevation, the superior vermiform process. In birds and in animals lower in the scale, this middle part of the cerebellum alone exists, and in animals it is the first part to be developed; moreover, in most mammals it forms a central lobe very distinct from the lateral portions. The hemispheres are separated behind by a deep notch. The upper vermiform process, though slightly elevated, is not marked off from the hemispheres, so that the upper surface of the organ, which is somewhat flattened in the middle and sloping downwards, at each side, is uninterrupted. Below, the hemispheres are convex, and are separated by a deep fossa named the vallicula, which is continuous with the notch behind, and in it the inferior vermiform process lies concealed in a great measure by the surrounding parts. Into this hollow the medulla oblongata is received in front, and the falx cerebelli behind. The greatest diameter of the organ is tranverse, and extends to about three and a half or four inches. Its width from before backwards is about two or two and a half inches; and its greatest depth is about two inches, but it thins out towards its lateral borders. The surface of the cerebellum is everywhere marked by deep, closely set transverse fissures which extend a considerable depth into its substance. One of these, the great horizontal fissure, divides the cerebellum into an upper and lower portion, corresponding in fact to the upper and lower surfaces, in each of which there are several lobes."

The internal arrangement of the grey and white nervous matter composing the cerebellum is well seen in the accompanying slide. The central part is composed of white nervous matter which sends out divergent and gradually thinning layers into the interior of the laminae, larger and smaller, the grey nervous matter forming everywhere the outer covering. In consequence of this arrangement of white and grey matter sections of the cerebellum *crossing* the laminae present a foliated appearance named *arbor vitae*. This appearance is seen in any vertical section, but is most conspicuous in a section which passes through the median plane, where the relative quantity of the central (white) matter is small.

The foliations are arranged somewhat pinnately, the sections of each primary lamina having those of secondary laminae clustered round it, like leaflets on a stalk. *The main branches of the white* (central) *nervous substance, or groups of them correspond with the lobules* which, though we have not mentioned it, receive names according to their shape, or appearance, or position, or fancy of the anatomists who have described them.

"The outer or grey nervous matter, also called cortical, is composed of two distinct layers, having between them at their junction a layer of cells, called the corpuscles of Purkinje. Outside the whole is the pia mater, whose blood-vessels pierce the nervous substance. The two layers are named respectively

The outer layer,
The inner layer.

The outer layer consists of a delicate matrix, containing cells and fibres. Most of the *fibres* have a direction *at right angles* to the surface; the greater number of them are the processes of the large (Purkinje's) cells. The *cells* of this outer layer are granule-like bodies, some very small, and belonging, probably, to the matrix, others somewhat larger, and probably nervous, with processes extending from one or more sides. Some of the corpuscles are connected with the processes of the large cells of Purkinje. The inner part of the layer, contiguous to the cells of Purkinje, contains nerve fibres running *parallel* to the surface.

The inner layer, that next the medullary centre, consists of granule-like corpuscles, imbedded as close groups in a gelatinous matrix, which contains also a plexus of fine nerve fibres. The corpuscles of Purkinje dip into and are, therefore, almost surrounded by these granule-like corpuscles.

The corpuscles of Purkinje lie, as we have said, between the outer and inner layers of the grey cortex. They are mostly flask-shaped, with their longer axis at right angles to the surface. Processes extend from them into both the inner and outer layers; the outer processes being by far the larger and more conspicuous, branching and dividing as they approach the surface. The inner process, after passing into the granular layer, becomes the axis cylinder of a nerve fibre, therefore it is fine and undivided.

The white (central) nervous centre of each lamina consists of nerve fibres arranged in parallel or interlacing bundles, which pass from the central or white matter of the hemispheres, etc., and appear to turn obliquely into the cortical grey substance. These disappear in the granule layer and are believed to be continuous with the axis-cylinder processes of the corpuscles of Purkinje, but some anatomists are of opinion that they arise in part by the union of the fine fibres of a plexus in the outer layer."

Mode of Preparation.

The cerebellum of a human being, cat, dog, or rabbit may be taken and cut into pieces half an inch square. These pieces are then to rest on cotton wool on the bottom of a jar filled with three parts of Müller's fluid and one of common alcohol freshly mixed, but allowed to cool before use. Keep in a cool place during hardening. Renew the fluid in twenty-four hours, then in seven days. The pieces should be left about twenty-one days in this mixture altogether. They are then to be transferred to a large quantity of a 2 per cent. solution of bichromate of ammonia for another fourteen days to complete the hardening. Now wash away all the preserving fluid by repeated changes of clean cold water and transfer to common alcohol.

Sections are to be made *across* the folia or leaflets either by freezing or imbedding. The latter is preferable if very thin sections are required, because if freezing be employed the leaflets are apt to fall asunder, and the pia mater also gets torn away. The sections are then to be stained in an alcoholic solution of anilin blue-black make as follows :—

Take of

Anilin blue-black 1 decigram.
Distilled Water, 4 c.c.
Rectified Spirit, 100 c.c.

Dissolve the dye in the water thoroughly, then add the spirit and filter. This is to be kept in a well-stoppered bottle till required.

The sections are to be stained in the above, then cleared and mounted in balsam in the ordinary way.

BIBLIOGRAPHY.

We have largely used Quain's Anatomy, Vol. ii. in the above description, where a full account will be found, accompanied by excellent wood-cuts. Klein's elementary histology (Cassell and Co.) also contains a clear, concise account.

Fig 1

Fig 2

J. W. Watson, del. et lith.

HUMAN CEREBRUM

(after Klein.)

Watson & Son, Lith. 313 St. Charles St. Toron.

HUMAN CEREBRUM.

Vertical Section of the Grey Matter of a Cerebral Convolution T.S. × 150 diam.

Etymology.—Arachnoid (ἀράχνη, a spider's web, εἶδος, like) Arcuate (arcus, a bow). Corpus callosum (corpus, a body and callus, hard). Dura mater (mater, mother; durus, hard).

General Description.

The largest part of the human encephalon is called the cerebrum, and extends from the fore-head to the back of the head, also from one side of the head to the other; the depth—roughly speaking—is from the top of the head to a line drawn round the head through the eyes across the temples and through half the ear-lobes, which would become a complete band meeting high up on the neck. In other words the cerebrum is the brain as seen from the outside, so to speak.

The cerebrum is composed of two cemetrical halves which nearly touch. The two halves together form an ovoid mass flattened on its under surface, having the front end the small and the larger end behind. The two halves, though nearly touching throughout their course, are separated by a very deep cleft called the longitudinal fissure. Each cerebral hemisphere, therefore, has three surfaces: a *convex* surface in contact with the cranial vault, a *flat* surface which helps to form the longitudinal fissure, and an *irregular* under surface which mostly lies on the floor of the cranial vault.

The two large hemispherical masses we call the cerebrum are separated, as we have said, by the longitudinal fissure, but in the middle this fissure is not complete. A large transverse mass of white brain-substance links the two cerebral hemispheres together. This transverse mass of white brain substance is called the *corpus callosum* or great commissure.

The surface of the hemispheres forms an arch corresponding to the top of the head; the outer portion of the cerebrum or surface is not smooth like an egg, but is composed of eminences called *convolutions*, and depressions called *sulci*. Besides the ordinary *sulci* there are deeper

ones called furrows, which divide each half of the cerebral mass into *six lobes*. These lobes from their position or function are called—

1.—Frontal.
2.—Parietal.
3.—Occipital.
4.—Temporal.
5.—Central.
6.—Olfactory.

At the present day it is of the first importance for a doctor to be able to define pretty accurately the boundaries of these lobes. For our present purpose, we may say roughly that the frontal lobe occupies the front or fore-head; the occipital lobe occupies the back of the head; and the parietal lobe the intermediate space, and so forth. The reason the lobes are so important will be described presently.

The interior of the cerebrum is studied by paring away successive layers horizontally. The first cut displays the internal *white* matter of each hemisphere, surrounded on all sides by the *grey* matter. This is called *centrum ovale minus*. A second section on the same level as the top of the *corpus callosum* discloses a still greater area of *white* matter called *centrum ovale majus*. We now see that the white matter of the hemispheres is connected by the *corpus callosum* itself *white* matter. A third layer removed discloses the hollow space in each hemisphere called the *lateral ventricles*. On the floor of each ventricle are several structures: the most noted being the two large masses of *grey* matter, the greater part of each being imbedded in the *white* substance of the hemisphere in which it lies. These large ovoid masses are called the *corpora striata* or *ganglia* of the *cerebral hemispheres*.

The brain and spinal cord being continuous structures are covered by continuous membranes, three in number:—

1.—The dura mater.
2.—The arachnoid.
3.—The pia mater.

The dura mater is a very strong, dense, inelastic, fibrous tunic, which adheres closely to the inner surface of the bones of the skull and forms their internal periosteum, the adhesion being more intimate opposite the sutures, but within the vertebral canal, *i.e.*, as it surrounds the spinal cord it forms a *loose* sheath. The dura mater within the skull is doubled upon itself and thus is enabled to leave the bones and return to them at the same place. Such a duplication is called a process. There are three such processes or partitions, namely:—one running lengthwise from the forehead to the back of the head in the middle line, which dips down and separates the two hemispheres of the cerebrum: a second has a horizontal direction, stretching *across* the back part of the skull between the cerebrum and cerebellum: and a third which, like the first, is vertical, and separates the two hemispheres of the cerebellum. By means of the dura mater, therefore, the four large masses (2 cerebral, 2 cerebellar hemispheres) which form the brain, practically, are partitioned off, and enclosed with much firmness, *but without pressure*.

The arachnoid membrane invests the brain and cord closely, but does not dip into the *sulci*. It is a delicate membrane, consisting of fine fibrous tissue bundles interlacing with each other. The intervals between the spaces are filled up by delicate membranes, composed of expanded cells.

The pia mater is a delicate highly vascular membrane, closely enveloping the surface of the convolutions and dipping into the sulci of the cerebrum. From its inner surface numerous minute blood vessels penetrate the brain substance. The same also obtains in the case of the cerebellum. Around the cord the pia mater is thicker, firmer, and paler (from being less vascular) and more adherent to the nervous matter of the cord.

It will be seen then that the membranes of the brain and cord have three distinct offices:—the dura mater mechanically supports; the arachnoid secretes serous fluid, which also acts as a packing agent; and the pia mater supplies the means of life.

The Cortical or Grey Matter.

Our section and drawing give a representation of the grey or cortical matter which caps the convolutions and dips into the sulci of the cerebrum. It contains cells and fibres embedded in neuroglia, with numerous blood vessels which pass vertically inwards.

The cells of this cortex are arranged in layers; the most external layer is narrow and forms about the $\frac{1}{10}$th of the whole thickness of the grey cortex. It is composed chiefly of neuroglia and contains a few small cells with fine processes, probably not of nervous character. A few medullated nerve-fibres occur in it forming a thin superficial white stratum immediately underneath the pia mater. The next layer of nearly the same thickness is characterised by containing a large number of small nerve cells mostly pyramidal with branching processes. The third layer is of paler tint and much greater thickness. It contains pyramidal branching cells, large and small, arranged as above described, with the pointed extremities towards the surface of the convolutions, and separated into groups by the bundles of radiating nerve fibres. The inner portion of the layer, in which the cells are larger and the separation into groups more distinct, is sometimes described as a separate layer. The fourth layer is narrower, and contains many small irregularly shaped corpuscles, round or angular, with fine processes placed irregularly and less distinctly separated into groups. The fifth layer, of greater width than the last, and blending more or less with it, is composed of fusiform and irregular cells. The fusiform corpuscles have a definite arrangement, being placed for the most part vertically at the summit of a convolution, but in the sulci, parallel to the surface, where they correspond in direction to the arcuate fibres passing from one convolution to another; they are said to be connected with these fibres. Beneath the last layer is the medullary centre with which it gradually blends.

The cells are of various forms and sizes, many of them have numerous processes. Some of these branching cells are irregular in form and position, but the majority are more regularly pyramidal in shape, with the apex of the pyramid turned towards the surface of the convolution. The average size of the larger pyramidal cells is the $\frac{1}{1800}$inch in diameter at the base, and each has a rounded nucleus having an average diameter of a $\frac{1}{2500}$inch. They generally contain a little yellowish pigment. The cells often appear to lie in distinct cavities in the grey matter, but it is uncertain if these are natural or produced by shrinking after death. The process from the apex of each cell may be traced for some distance towards the surface of the convolution, giving off one or two branches as it passes outwards. The undivided axis-cylinder process probably arises from the centre of the base of the cell. The processes of these cells, as well as the body of the cell itself, possess a distinct longitudinal striation. The smaller angular corpuscles are also provided with branches which run in various directions and probably unite into a fine network. Rounded cells, having no visible processes, also occur.

The fibres radiate from the white centre of each convolution in all directions into the grey cortex having a course for the most part perpendicular to the surface. In passing through the grey substance they are arranged in bundles about the $\frac{1}{1800}$ inch in diameter, and thus separate the nerve cells into elongated groups and give the section a columnar appearance.

Mode of Preparation.

If possible, we ought to obtain a portion of human cerebrum, and choose a frontal convolution.

It is to be prepared like the cerebellum, as described in the last histological number, and stained with aniline blue black, and mounted in Benzole Balsam.

BIBLIOGRAPHY.

The second volume of Quain's Anatomy—from which we have largely quoted—contains a very concise description, explained also by the use of excellent wood-cuts.

If the reader wishes to know how very important a matter it is to know the exact locality of the various convolutions, he should procure Ferrier's work on the Brain (Macmillan and Co.) Ferrier has found by applying the interrupted current to certain given areas on the convolutions of the brains of dogs and monkeys, that he can cause the movements of various sets of muscles to a certainty thus:—By applying the current to one area he causes the hind leg to advance as in walking; to another given area, the tail is wagged; to another, the shoulder is elevated; to another, the lip is elevated, and the nostril dilated; also numerous other combined muscular movements, can be produced with precision and certainty. When spiculæ of bone or matter press on these known areas, that area the surgeon determines by the results produced; he trephines, and is able thus to relieve it of the offending substance which would cause death if allowed to remain. This terrible knowledge has been obtained by taking away the skull-cap of numerous dogs and monkeys, and as it has been the means of saving numerous human lives, and will doubtless restore to his family many a human bread-winner in the future.

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SECTION I.

ANIMAL HISTOLOGY.

PAGE 9.—l. 5 from foot; for "cartilage is tissue" read "cartilage is a tissue."

PAGE 11.—l. 2 from foot; for "the white corpuscles is not," &c., read "the white corpuscle is not," &c.

PAGE 25.—l. 11 from foot; for "their areolar character of is shown" read "their areolar character is shown."

PAGE 29.—l. 14 from top; for "the proportion of carbon and hydrogen" read "the proportions of carbon and hydrogen."

PAGE 44.—l. 13 from foot; for "solution of aniline blue black make as follows" read "made as follows."

PAGE 45.—l. 15 from top; for "cemetrical" read "symmetrical."

PAGE 48.—l. 2 from foot; for "and as it has been the means" read "and it has been the means."

E D del. ad. nat. J.W. Watson del. et lith.

PINUS SYLVESTRIS

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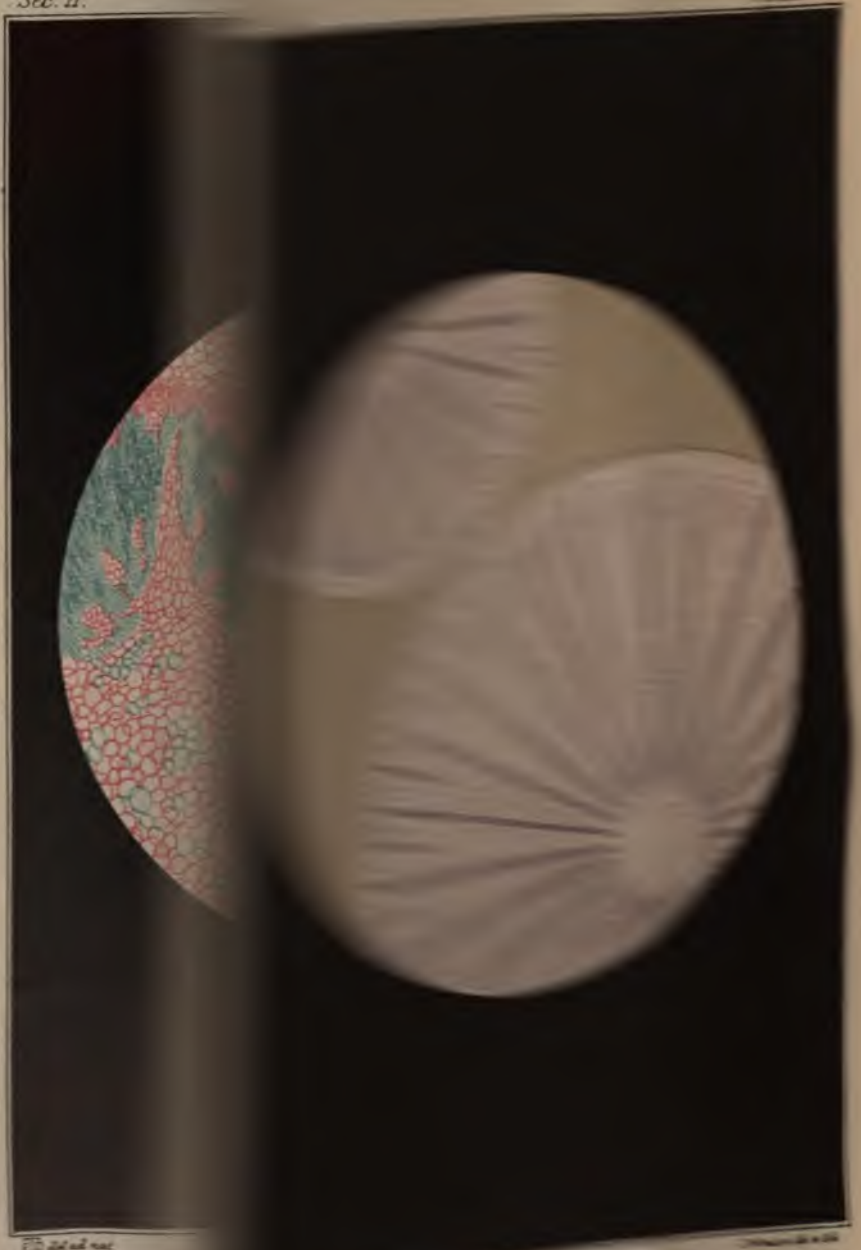

ARACHNOIDISCUS EHRENBERGÜ
(recent.) Monterey Bay
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STUDIES IN MICROSCOPICAL SCIENCE.

SECTION II.—BOTANICAL HISTOLOGY.

CHAPTER I.

THE MORPHOLOGY OF THE CELL.

Modern vegetable histology may be said to have had its origin in SCHLEIDEN'S[1] enunciation, that the tissues of plants are built up of cells. Literally, histology[2] is concerned with the general morphology of tissues; it is only since the introduction of the microscope, as an instrument of power in anatomical research, that histology has ranked as a science which deals exclusively with *minute* structure. The cellular nature of vegetable tissues, especially of such as are likely to be examined by the beginner, viz., those of the higher plants, is apparent at first sight when viewed with even a very low power of the compound microscope, but it must be borne in mind that when SCHLEIDEN framed his theory, he laboured under technical methods of manipulation of a very primitive character; his discovery, therefore, marks an epoch in the history of botanical science.

THE CELL THEORY.—The foundation thus laid by SCHLEIDEN in one department of biology, was speedily extended to include the more obscure nature of animal tissues, and SCHWANN immortalised his name at this early period by the statement that, amongst animals, "*there is one universal principle for their development, and that principle is the formation of cells*,"[3] and this dictum is the

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[1] *Müller's Archiv*, 1838.

[2] Gr. ἱστος, web, tissue; and λόγος, discourse. Also written *Histiology*, from Gr. ἱστίον, tissue.

[3] *Mikroskopische Untersuchungen*, 1838; Syd. Soc. Transl., London, 1847. p. 165.

more remarkable from the fact that it was arrived at when the known methods of investigation at the command of the histologist, were such as to involve the utmost difficulty in research, and when the optical means at his disposal were often quite unreliable. Even at the present time, when microscopes and the methods of research have reached so high a standard, that it may with justice be doubted whether they can be much improved, it is sometimes most difficult to imagine that certain tissues are built up of elementary units, which preserve an individuality more or less independent of each other. That such is in reality the case does not now admit of any dispute; all tissues that have hitherto been examined microscopically prove the universality of SCHWANN'S doctrine.

But, whilst investigating the morphology of tissues, SCHWANN attempted to account for their origin; and the imperfect means at his command led him erroneously to believe that cells may arise *de novo* by a sort of precipitation in a structureless pre-existing substance which he called a *cytoblastema*.[1] ROBERT BROWN,[2] in 1833, drew attention to the *nucleus*, which had been figured as early as in 1802, by F. BAUER,[3] and SCHWANN[4] applied the term *nucleolus*, to the smaller denser body within the nucleus which had already been observed by SCHLEIDEN, but here again he fell into the error, that the formation of the cell commences within the cytoblastema by the separation and coalescence of particles which form the nucleus; around this centre an analogous precipitation produces the cell-wall, and between these two, the so-called *cell-fluid* accumulated. BARRY,[5] and subsequently GOODSIR,[6] protested against this theory of cell formation, and established, that all cells arise through regular descent from pre-existing cells.

The second great advance in histology was the discovery, by H. VON MOHL, of *protoplasm*[7] in the cells of plants, and here, as in the former instance, its animal analogue was shortly afterwards identified by COHN, REMAK, and others. But now a complication ensued, which somewhat retarded the progress of histological science; SCHWANN defined a cell as essentially—a nucleus and a cell-wall; to the cell-wall he attributed the power to elaborate and modify the cell-contents, and this view of the case was all but universally accepted, when LEYDIG[8] pointed out the error by showing that certain cells, *e.g.*, pus, mucus, etc., do not possess cell-walls, and that the cell-substance, or protoplasm, is the essential portion,

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[1] Gr. κύτος, cell; βλαστημα, bud, sprout.

[2] *Miscellaneous Botanical Works*, Vol. I, p. 512.

[3] In the cells of the stigma of *Bletia Tankervilliæ*; also figured in 1830 by MEYEN in his *Phytotomie*.

[4] *Schwann and Sch'eiden's Researches*, p. 3.

[5] *Phil. Trans. Roy. Soc.*, London, 1838—39.

[6] *Anatomical and Pathological Observations*, Edinburgh.

[7] *Ueber die Saftbewegungen im Inneren der Zellen, Bot. Zeitg*, 1846, p. 73.

[8] *Handbuch der Histologie*, 1856.

from which the cell-wall is merely a secondary derivative, and is produced either by condensation of, or secretion from, the protoplasm, or body of the cell. Goodsir's researches reverted to the importance of the nucleus, and, from its frequent division preliminary to cell multiplication, he averred that it must be the *germinal centre* of the cell. Such was the condition of things, when, in spite of Leydig's demonstrations, Virchow[1] insisted on the cell-wall as an indispensable moiety, and it was not until 1861 that Beale[2] and Max Schultze[3] succeeded in abolishing the primary importance of the cell-wall once more. The cell was now considered to be essentially *a nucleated mass of protoplasm*.

Vegetable histology again took the lead in the important announcement by Brücke,[4] that many cells, *e.g.*, the cells of *Fungi*, are devoid of nuclei; and once again it was speedily discovered that the nucleus is not always present in the cells of animal tissues. Beale demonstrated a minute non-nucleated colourless corpuscle in the blood, Schultze described his now well-known *Protamœba porrecta*, and Haeckel laid the foundation stone of the order *Monera* (the members of which consist of formless masses of protoplasm which have been banded about from the vegetable to the animal kingdom), in his then unique *Protogenes primordialis*. And now, with the rapidly increasing variety of forms, a complication arose, of a most serious character, which threatened to throw histological nomenclature into chaos. Since Leydig, Remak and Schultze had applied the term *cell* to a *nucleated* mass of protoplasm, Haeckel sought out a new name for his *non-nucleated* form in its Hellenic equivalent — *cytode*, and the term came into general use. But this manufacture of terms would need to be carried to a much greater extent to be at all adequate, and new names would have to be invented for such things as the cells of the periderm of the higher plants, which have become thoroughly suberised, the corky change having supervened to such an extent as to leave no trace of anything but a thickened cell-wall, alike impervious to air and water; or the lignified cells and cell-fusions of woody-tissue, upon whose passive mechanical properties the healthy continuance of the life of most of the higher plants depends, would have to be described by other names, which indicate that they consist merely of thickened cell-walls, from which both cell-contents and nuclei have disappeared.

A way out of the difficulty has been pointed out by Rutherford,[5] who maintains that the classical term *cell* should be retained to indicate any one of three things:—

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1 *Cellular Pathology*, translated by Chance, 1860, p. 12.
2 *The Structure of the Elementary Tissues*, London, 1861.
3 *Müller's Archiv*, 1861, p. 18; *Das Protoplasma*, Leipzig, 1863.
4 *Die Elementar. Organismen. Sitz. d. k. Akad*, Wien.
5 *A Text-Book of Physiology*, Edinburgh, 1880, p. 30. Here the terms *protoplast* and *periplast* are used as synonymous with cell-contents and cell-wall respectively. They point to the relative positions of the parts of the cell.

1°. A simple protoplast, with or without a periplast.
2°. A nucleated protoplast, with or without a periplast.
3°. A periplast.

Examples of the first variety of the cell are to be found only amongst the lower orders of plants, and most abundantly in that debateable group, half plant and half animal, the *Regnum Protisticum* of HAECKEL. In certain stages of some of the lower cryptogams, as amongst the *Myxomycetes, (e.g. Didymium, Physarum, etc.,) plasmodia*, or creeping masses of protoplasm are formed which are not unlike some of the lower forms of animal life ; their true plant nature has, however, been determined through a study of their life-history which is essentially analogous to that of other undoubted plants, the well-known *Pandorineæ*.

But sooner or later most vegetable cells develop cell-walls of cellulose and thus come under the second category ; instances are to be found in almost all orders of plants ; amongst the *Fungi* the unicellular forms as in the common yeast *(Torula* or *Saccharomyces Cerevisiæ)*, or the multicellular mycelial varieties, *(Agaricus, etc.)*, possess only cell-walls and cell-contents without nuclei ; the tissues of most of the higher plants possess nucleated cells, and the cell-walls are usually held together by an interstitial material, the significance of which will be fully discussed hereafter. In some of the *Algæ*, this intercellular substance becomes enormously developed and modified, so much so, that it seems to be entirely distinct from the cellular elements, which appear to lie embedded in it.

The epidermal tissues of plants, the outer protective coverings of their organs, and the inner lignified elements which either remain distinct and truly cellular, or become fused to form vessels or ducts, are all examples of the third variety of cell, viz., a cell-wall from which cell-contents have vanished. Although the lignified cells of woody-tissue have lost their protoplasmic contents, they cannot be looked upon as dead cells, because in the latter case a certain amount of natural decay and disintegration would take place in the exposure to which they are subjected ; they are not dead although they are functionally passive. On the other hand, certain of the cells which lose their contents and are eventually reduced to mere cell-walls, as in the outermost layers of the bark of trees, are practically dead ; they are to be compared to the desquamated horny epithelium of the skin of the higher animals.

All these considerations taken together point to the diversified morphology of the cell, but they also show, that its primary and essential part is the protoplasm, or, as HUXLEY[1] has aptly termed it, the "*physical basis of life.*"

[1] *Lay Sermons Review, etc.*, London.

THE VARIABILITY OF FORM IN CELLS.—From what has already been stated with regard to the nature of the cell, it naturally follows that in form cells present innumerable differences, and that these are dependent upon the relative development of certain parts.

It has been pointed out that the protoplasm is the primary and essential part of the cell, for without it the growth of the cell ceases, and its multiplication becomes impossible; from it the cell-wall is produced, and as the latter becomes larger and thicker, the protoplasm lessens relatively in quantity, and, at the same time, contributes to the production of a contained fluid, the cell-sap, until, finally, it is entirely absorbed, and nothing save a dead cell-wall remains. This is the condition of things to be observed in such a simple form as the common yeast plant *(Saccharomyces Cerevisiæ)*.

In order to understand the facts just stated, the development of the yeast should be studied. The first or gemmiparous condition may be observed thus:—

Procure some brewer's yeast[1], and sew a small quantity in a vessel containing the following modification of PASTEUR'S fluid[2], and keep it in a warm place.

Water (H_2O)	8576	parts
Cane Sugar ($C_{12}H_{22}O_{11}$)	1500	,,
Ammonium tartrate ($C^4H_4(NH_4)_2O_6$ ...	100	,,
Potassium phosphate (KH_2PO_4)	20	,,
Calcium phosphate ($Ca_3P_2O^8$)	2	,,
Magnesium sulphate ($MgSO^4$)	2	,,
Oxygen (O)		

Very soon the fluid will begin to froth up, and show that the active life of the plants has commenced. To make out the peculiarities of the cells a high power of the microscope (about from 700-1200 diameters) will be required. A small drop of the fluid should be placed on a glass slip, covered with an extra thin cover glass, and examined. The cells will probably be found in every stage of development. Some are isolated, whilst others are connected in groups. Amongst the isolated forms every variety may be discovered by the use of reagents, but it is not always easy to find a group which shows every stage in the development by budding of the yeast; for this purpose it may be necessary to keep the plants under continued observation for several days in a moist chamber.

1This is commonly known as *barm*, (A.S. *beorma*; Ger. *barme*), and may be procured from any brewery, or from most public houses.

2PASTEUR himself used yeast ash, but the fluid given above is easier to prepare, contains all the constituents of the ash, with the addition of the ammonium tartrate and sugar, and serves quite well for all ordinary purposes. (See PASTEUR, *Comptes Rendus*, Paris, T. xlvii, p. 1011).

Magenta solution[1] is perhaps one of the most useful in revealing structure here. A small drop of the dye should be placed on the glass slip at the edge of the covering glass, and drawn through the field of view by means of a piece of blotting paper applied to the opposite edge of the cover. It will be noticed that some of the cells will not stain or only become faintly tinged when a strong solution of the dye is used; these are the old cells from which the protoplasmic contents have disappeared, and are therefore merely cell-walls. Other cells become deeply stained, and it may be observed that the smallest of them, especially those which exist in the form of buds, imbibe the dye most readily of all.

These very young buds are apparently devoid of any cell-wall, but gradually, as they grow older and larger, a very thin membranous envelope may be discovered. The next stage shows the envelope plainly, and at the same time the centre of the protoplasm becomes hollowed out,—a *vacuole* is produced in which the cell-sap accumulates. That it is indeed a vacuole, and not a nucleus, or denser portion of the protoplasm, may easily be proved. When the object is living, and unstained, a slight variation in the fine adjustment of the microscope causes it to become alternately bright and dark, thus showing it to be a space of some sort in the protoplasm. On the introduction of the magenta or other stain, this effect is only heightened; whereas if it were a nucleus, as rarely happens to be the case, it would become darkly stained, and remain so under a slightly varying focus. Again, when such reagents as a strong solution of potassium hydrate or acetic acid are used, it disappears altogether; the reaction of these fluids being solvents of protoplasm, so that if it were a denser portion of protoplasm, or a nucleus, it would at first resist the action of the reagent and stand out in relief; but, as it is only a space in the protoplasm, it vanishes.

These confirmatory tests prove it indisputably to be a *vacuole*, and that the space is filled with fluid is shown by its contour which is strikingly different from that of a bubble of air; the outline of the latter is much more marked because of the greater differences in the refractive indices of the two.

As the cell grows older the protoplasm becomes more and more vacuolated, and the cell-wall increases in thickness; either the vacuole becomes larger, or several are produced. Finally, the protoplasm gradually lessens, adheres to the sides of the cell-wall, and eventually disappears altogether. The cell now consists of a mere envelope, its fluid contents are all absorbed, and it decays,—a sign of the absence of life.

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[1] To make this solution, dissolve 1 decigr. of crystallised magenta (roseine) in 160 cubic centimetres of distilled water: add 1 cub. cent. of absolute alcohol. Keep in a well-closed bottle. (HUXLEY and MARTIN'S *Elementary Biology*, 1879, p. 269.)

Although the protoplasm can be observed clearly through the cell-wall, it is otherwise demonstrable. If a small pad of blotting paper is placed upon the top of the covering glass of a specimen which has been stained, and gently tapped with a pen-holder or knife-handle, some of the crushed cells will show the protoplasm clearly extruded from the cell-wall. The action of iodine solution on the cells is of value to show that they do not contain starch, otherwise they would show blue colourations. Osmic acid is useful also to reveal particles of a fatty nature in the protoplasm.

If now a small piece of dry German yeast is procured from a baker, shaken up in a large test tube, and allowed to stand for about an hour, the yeast cells will have separated sufficiently to enable them to be spread upon thin slices of a freshly-cut potatoe; this can be done with a camel's hair brush. The slices thus treated should be laid upon a plate of plaster of Paris, this, in its turn, upon sheets of wetted blotting paper; or a blotting pad alone may be used, and the whole placed under a bell-jar. The blotting-paper must be kept continually moistened with water. At the end of the eighth or ninth day, a fine scraping may be examined under a high power of the microscope, and will then show the process of development by *ascospores* or *endogonidia*. This process may also be observed by sewing ordinary barm in plain drinking water for a few days.

The protoplasm of some of the cells will be found to sub-divide internally into four rounded parts. At first these ascospores are devoid of any cell-wall; they are merely surrounded by the common envelope of the parent cell. But as they develop, each particle secretes a cell-wall, the original envelope bursts, and they are liberated to proliferate by gemmation in the manner already described.

In both instances the buds or young cells began life as simple undifferentiated masses of protoplasm, to these envelopes were superadded, and vacuoles developed. Lastly, the protoplasm, or seat of growth, vanished gradually, until its total disappearance heralded death and decay. These record some of the simplest cases in the development of the individual cell, the external form of the cell varies but slightly during its increase in volume.

Amongst the higher plants the growth in size of a cell from a simple mass of protoplasm is usually accompanied by a variation in form whereby an originally spherical or ovoid cell may ultimately become polyhedral, elongated, prismatic, tabular, or even branched. These parts of the cell (its protoplasm and wall) may become so modified that they subserve the special requirements of the various parts of the economy of the plant.

In the reproduction of the common bladder-wrack (*Fucus vesiculosus*) an excellent example is afforded of a simple mass of protoplasm

becoming clothed with a cell-wall, developing places for cell-sap and eventually growing into a large complicated system of cells, some of which perform special functions, but are nevertheless simple enough to be compared with the primitive yeast plant on the one hand, and complex enough to foreshadow the various organs of the highest plants on the other.

Certain branches of these large sea-weeds possess peculiar enlargements, studded all over with small spores, each of which opens into its respective *conceptacle* as these are termed. If thin slices of these conceptacles are examined in salt water, or normal saline solution, those obtained from the female conceptacles will be seen to contain certain large cells termed *oogonia*. Each oogonium consists of a cell-wall filled with a finely granular protoplasm, which in its young condition seems to be quite structureless, but ere long the contained protoplasm breaks into eight nearly equal parts, known now as *oospheres*. The eight *oospheres* entirely fill the cavity of the oogonium, and as they are closely pressed against each other, their external shapes are polygonal. The outer wall of the oogonium now splits, and the eight oospheres are expelled, surrounded by a very thin membrane, which once formed the inner coat of the oogonium; the membrane becomes distended by the absorption of water, and each of the oospheres now assumes a spherical form. They thus collect in numbers at the mouths of their conceptacles, while the fertile branches are lying outside of the water when the tide has receded. On the rise of the tide, the thin membrane bursts and liberates the spherical oospheres which are still devoid of any cell-wall. At the same time other small bodies termed *antherozoids*, are set free from the male conceptacles, and come into contact with these naked oospheres. Profound changes now go on which result first of all in the formation of a transparent cell-wall, and the cell fixes itself to some external body, and begins at once to germinate. This it does not do after the manner of the yeast plant, but the whole of its cells remain organically united. The fixed portion produces a root-like organ of attachment, whilst the free end develops leaf-like branches, on which conceptacles are afterwards produced.

The cell-walls in these plants undergo a peculiar change which is common to most of the *Algæ*, and which is worthy of attention here. If a thin section of the thallus of the plant, preferably taken from its thickened stem-like portion, is examined under a power of about 150 diameters, it is seen to consist of a number of cells which vary in form in different parts of the structure, but which agree in producing cell-walls of a two-fold nature. Each cell is surrounded by a wall which evidently belongs to itself, but the interspaces between the cells are filled with a material which differs from the ordinary cell-walls in that it is distinctly mucilaginous. There is reason to believe that it is produced by a separation and subsequent coalescence of particles derived originally from the primary cell-walls

In some of the simpler forms of Algæ this jelly-like substance is often used for social purposes. For example, many unicellular individuals, like *Glœocystis* and *Palmella*, are able to live together in colonies, owing to the formation of stratified mucilaginous outer boundaries to their cell-walls. In many cases comparatively large "families" are formed, as in *Botrydina*, while in others the co-operative result may be the formation of a tube, or tubes, as in *Hormospora*, or a pear-shaped colony, as in *Apicystis*.

But the cell-wall in unicellular Algæ may suffer a perhaps still more marked and decided modification. The silicification of the cellulose membrane, weakly developed in some desmids, is carried to the highest possible perfection in the *Diatomaceæ*. These organisms varying infinitely in form, and, from actual invisibility to our eyes, to an appreciable size, are provided, as is well known, with flinty coats of mail, most wonderfully marked with faultless geometrical designs. The cases or *frustules*, as they are technically called, are two-valved, and as multiplication by division is by far the most common mode of reproduction in this group, and also as when the mature cell divides, an old half-frustule is retained by each daughter-cell, while a new silicious half-case is built on the free face of each separating rejuvenised mass, it of course follows that the two valves are of different ages. Each valve is made up of a back and an inturned hoop-like rim or girdle. The girdle of the old valve—often likened to the lid of a pill-box—slips over the girdle of the younger, the line of junction being descriptively known as the *suture*.

One of the disciform valves of the frustule of *Arachnoidiscus Ehrenbergii* [Plate 3] is selected to illustrate this differentiation of cell wall brought about in these, in other respects, simple organisms. The genus Arachnoidiscus is distributed in the seas of both hemispheres, and, according to Smith, Ehrenbergii is the only species belonging to Britain. The gathering from which the accompanying preparation was selected was taken in Monterey Bay, where the forms were found growing upon sea-weed.

The valve, although so extremely thin, is nevertheless made up of two distinct layers, as may be easily seen by using a good $\frac{1}{4}$ inch objective and focusing for the two levels. The outer layer is said to be of a remarkably tough, more or less flexible, acid-resisting, horn-like nature, while it is the inner coat alone that is impregnated with the silica. The two layers can, by prolonged boiling in nitric acid, be actually separated. It will be noticed that the inner layer is marked with lines of *puncta*, arranged both radially and concentrically, and that there is a central plain spot, the "pseudo-nodule" of descriptive writers. Around this pseudo-nodule may be seen a well-defined double row of "*puncta*," the individual markings of the inner circle being linear, while those of the outer are round. Respecting the outer layer of the valve, it is marked with radiating bars of equal length, springing from the circumference, and approaching, but not quite meeting, in the centre, their concentrically arranged terminations forming a boundary to the double row of markings surrounding the pseudo-nodule noticed above; while shorter and less conspicuous lines of unequal length alternate with

these more fully developed rays. To the somewhat striking resemblance in appearance of these markings to a spider's web the generic name of this Diatom is due.

In the higher types of vegetable life we meet with a greater diversity of form in cells. Here various systems of tissues are developed to subserve the physiological demands of the more highly specialised individual. A section through part of the body of any vascular plant will serve as an illustration. A young shoot of Scotch fir *(Pinus sylvestris)*, seen in cross section, has been selected (Plate 2). It presents, roughly speaking, five regions—(1) pith, (2) wood, (3) cambium, (4) cortex, (5) dermis. The cambium zone, immediately surrounding the green-stained wood, is made up of thin-walled elongated cells, growing closely together, each cell presenting a brick-shaped outline in transverse section. A magnification of 30 diameters is not sufficient to clearly define them, hence the drawing fails to show this region with satisfactory distinctness; but the preparation itself, examined under either a $\frac{1}{4}$ or $\frac{1}{8}$ inch objective will most clearly display it. These cells, during the life of the plant, are filled with protoplasm, and never (so long as they are members of the zone) advance beyond this simple torula-condition. Like Torula, they require, as food, constructive materials of an organic nature, and when so fed, also like Torula, they multiply themselves by a process of division. The daughter-cells so formed may advance towards no higher type of cell, as may be seen in the plates (lines in the section) of cellular tissue connecting the pith with the cortex.[1] But, on the other hand, these primitive cells may morphologically progress to a very special stage, as is exemplified by the tissue cells of the wood. Here, to give strength to the structure and for other physiological reasons, the cell walls are thickened from within, while, at the same time, to provide for the passage of liquids and gases in all directions, numerous spaces opposite to one another on contiguous walls are left unaltered, and the weak separating double membrane may eventually become absorbed, thus making a direct communication between neighbouring cells. These specialised cells (or *tracheïdes* as they are termed) may be well seen by making a thin *longitudinal* section of any of the *Coniferæ*, and after very carefully heating the slice in nitric acid and potassium chlorate for some time, if it is transferred to a slide and the cover glass gently tapped before examining, the "pits" will be very clearly observable.

The cells pushed outwards by the active cambium zone also become specialised in time, but as the changes in form and physical properties of this or similar regions will be the subject of future study, they need not even be broadly referred to now, as our present object is merely to indicate, in a general manner, the possible variability of form in cells.

Secretion-Cell.—Cells may become strikingly differentiated from neighbouring cells by becoming specialised to secrete from the circulating

[1]These rays being very narrow, are not shown in the drawing, but may be easily made out in the preparation under a ⅛-inch obj., as the cells have taken up the carmine stain.

sap materials which, in some cases at all events, may be looked upon as excrementitious matter. In the region of the wood in the section of Pinus, such cells may be discovered; they have taken up the carmine stain. With a good ¼-inch objective four nucleated cells lying in the same plane will be seen, and, owing to the withdrawal, or rounding off, of the cell-walls in each cell at the point where the edges of the four cells primarily met, a space is left which serves as a receptacle or passage for the material secreted by the cells. The material secreted by the Scotch-fir is a resin which may be of special use in cases of injury to the trunk or its branches, acting at once as a balm and protection to the suddenly exposed surface or wound. In the cortical region owing to the formation of a wider tube through repeated division of the four primary cells, wider passages occur; these are in communication with the wide resin passages of the leaves[1].

CELLS WITH SPECIAL CONTENTS.—Biennial or perennial plants, whose assimilating organs suffer destruction in winter, lay by during the season of activity, a store of assimilated food to be used in the following spring, for the purpose of nursing the buds through the critical period of nutritive dependence—that is, until the green leaves burst and spread themselves out to the air and light. The bulk of the reserve material is usually starch, but it may be some other substance, isomeric with starch, while always there will be mixed with it a small quantity of food of a protinaceous nature. This constructive material is stored away in cells, which must, of course, be thin-walled; while at the period of the in-filling, at all events, they must also contain working protoplasm.

To show these starch-containing cells, a section of the thick scale-leaf from the bulb of Crown Imperial *(Fritillaria Imperialis)* has been selected. The preparation is stained with logwood. Those cells that have not been cut into by the section knife, display a well-filled ca ity of variously-sized granules; while in some cells the old nucleus of the protoplasmic contents is still persistent. If thin sections are made from the fresh bulb, cells may be discovered in all stages from those containing nucleated protoplasm, with vacuoles, to the completely filled starch-bearing cells, as shown in the preparation. It will be found that those cells lying near the surfaces of the succulent scale-leaf will contain few or no starch grains, but will exhibit in a marked manner the ordinary contents of living cells. If the slice is treated with weak potash-water, the protoplasm may be seen dissolving away, leaving the large nucleus a very conspicuous object in the cell. It would be well to study the starch grains in a fresh specimen, either in section or isolated, by a process of maceration. In form they somewhat resemble one of the valves of the shell of a fresh-water mussel. At the narrower end lies the hilum, and, starting from here, concentric layers, alternately dark and light, may be easily observed by a proper adjustment of the light. The potash solution used above to display the nucleus, will cause these grains to swell so enormously, and at the same time render them so transparent, that they will become almost, if not quite, invisible.

[1]Vol. I. page 286—preparation 46.

In the preparation, other structures can be seen, namely, sections of the feebly-developed fibro-vascular cords that run up the succulent leaves, from the flatly-stunted stem at base of bulb, but as these have no present interest, they need not be described here.

Methods of Preparation, &c.

The gathering from Monterey Bay, California, from which the valves of "*Arachnoidiscus Ehrenbergii*," for the accompanying preparation were selected, is extraordinarily rich, in respect of the variety of forms it contains, yielding Arachnoidiscus Ehrenbergii (in profusion), Arachnoidiscus ornatus, Aulacodiscus oreganus, Eupleuria pulchella (in abundance), Isthmia nervosa, Hyalodiscus stelliger, Hyalodiscus subtilis, Triceratium arcticum (vars. a and b), and many small Naviculæ, Amphitetras, &c., &c. The Diatoms being in an exceptionally clean and perfect condition, growing parasitically upon an alga, the prolonged boiling in nitric acid, followed by sulphuric acid, which is ordinarily necessary, and from the effects of which the valves frequently become seriously abraded, was avoided and a perfectly clean and pure Diatomaceous residuum, easily freed alike from heavy sand and light floating particles, obtained by means of the following simple treatment. The alga having been placed in a large basin, in order to provide space for effervescence, many organisms containing lime being present, was left to soak for twenty-four hours in 5 parts water and 1 part hydrochloric acid—2 parts more of the acid were then added, and after the subsidence of the renewed effervescence, the mixture was thoroughly stirred for some time with a glass rod. The gathering was then carefully strained through very fine muslin, water being poured upon it with considerable force. The diatoms and finer sand, only, could pass through the muslin, which retained the coarse sand and all the flocculent matter, which it is so difficult to remove when the alga has been boiled together with the diatoms. The strained matter was then boiled in pure nitric acid for twenty minutes, some crystals of chlorate of potash being added during ebullition. The acid was entirely removed by repeated washings, and the gathering gently boiled for one hour (in a large test tube) in a very weak solution of bicarbonate of soda, and, lastly, repeatedly washed in distilled water. The diatoms, thus perfectly cleaned, were spread upon slips and dried, the finer forms being selected and transferred to covers, and mounted in balsam. Most interesting "strewed" slides may be made from such gatherings by allowing a drop of the cleaned material to fall (from a *heighth* in order to spread it evenly) on to a covering glass from a glass tube of small diameter, and mounting the forms so spread in balsam or "dry." The alga itself, also, if perfectly freed from salt by prolonged soaking in water, repeatedly changed, will yield beautiful slides either "dry" or mounted in balsam. In the latter case the alga must be placed in spirit after being removed from the water, and afterwards in turpentine, where it must remain for some days, in order that the air may be expelled from the frustules and replaced by the turpentine, before applying the balsam.

last month (October) from a narrow, sluggish water ditch, in a boggy slope on Wimbledon Common, Surrey. The bottom of the ditch was filled to a considerable depth with peaty matter, and, in certain places, the thin sheet of water was fairly covered with this Micrasterias, some as isolated individuals, others associating themselves in small colonies, or in broad, deep masses, formed almost entirely of these cohering organisms. In the latter case the lower surface of the green mass was generally attached to, or even mixed with, the soft brownish peaty matter at the bottom of the ditch, making anything like a clean "dip" a matter of some difficulty. This species of Micrasterias is, as may be seen, a flat, obtusely-oval, much denticulated organism, the mature individual presenting an all but complete separation into two similarly shaped sub-hemidiscoid cells, each half being partially separated from its closely opposed fellow by two deep, straight, and opposite clefts; the cell cavities being united merely by a median, hollow, narrow isthmus of cell wall substance left as a junction collar between the two bilaterally symmetrical half cells. The cell is filled, or almost so, with a densely granulated, intensely green protoplasm, often containing in addition, numerous, scattered, variously sized, oily-looking globules. Each half cell is deeply indented into five primary lobes, having the free, lateral faces in each case plane and closely applied. Each lobe (excepting the polar or terminal one in each half) is similarly indented, but not more than half so deeply as the last, while the secondary lobes so formed are themselves still again indented each by a shallow, wide, or wedge-shaped cleft forming the ternary and ultimate truncated denticulations of the little plant. The primary polar lobes are narrower, and only once indented while the sub-lobes are shorter than the secondary, but somewhat longer and broader than the ternary denticulations of the primary lateral lobes.

The endochrome does not, seemingly, fill the whole cell, but a clear margin, varying in breadth in different individuals, may be seen along the entire denticulated circumference of the cell. Widening at the polar regions, it here forms a comparatively large and distinctive vacuole-like feature; while a similar clear space, usually more or less round, exists in the central region—the isthmus above referred to—and its immediate surroundings.

Under a good ¼-inch obj., and by focussing exactly above the endochrome, and just below the cell-wall, in a living and vigorous specimen, a number of small, but variously-sized, clear-looking globules may be watched moving, usually with a more or less jerky motion to and fro between the clear space in the centre and the clear polar regions. The clear space at the poles usually contains a considerable number of these granules, and these are always in a very active free-moving state, owing, apparently, to the increase of space discovered here, after their obviously partially retarded movements over the surface of the endochrome. At the equatorial clear space the number of granules is never so large, neither are their movements ever so active as at the ends. Many granules, after journeying to this region, seemingly strike themselves against some part

CHAPTER II.

THE CELL AS AN INDIVIDUAL.

In Torula, as we have already seen, a single histologically simple cell may perform all those physiological functions that are necessary for the maintenance of life; and that under certain favourable external conditions (presence of warmth, moisture, suitable food, and oxygenated air) the cell will also grow, and in time increase itself by a process of budding.

It was shown that the natural food out of which the protoplasm of the light-shunning yeast plant was able to construct new protoplasmic material was sweet juices, the nutrient constituents of which had been elaborated through the vital actions of other plants, and that, therefore, being itself unable to manufacture such ternary organic compounds as sugar or starch, it is hence wholly dependent upon the energy of pre-existing life for the greater bulk of its food supplies.

In unicellular plants, having the power of producing chlorophyll, we have individuals differing, physiologically, very widely indeed from the torula type. These green cells have the power of producing, through agencies at work within their own cell walls, the ternary compounds, so essential for the construction of protoplasm, and the raw materials out of which such compounds are manufactured are water (H_2O) and carbonic acid gas (CO_2). Provided, then, with such simple substances in an available condition, and enjoying a suitable warmth together with a proper amount of light, such cells are able to "assimilate" within their chlorophyll bodies either starch or some other material chemically allied to that organic compound. Cells such as these, therefore, may be looked upon as Nature's laboratories, wherein inorganic molecules are transformed into organic through the combined actions of many converging energies focussed within the chlorophyll-bearing corpuscles; while, also, such green cells may be looked upon as pioneers of life, elaborating not only necessary material for the use of their own protoplasm, but making life—fungal and parasitical existence generally as well as animal—really possible.

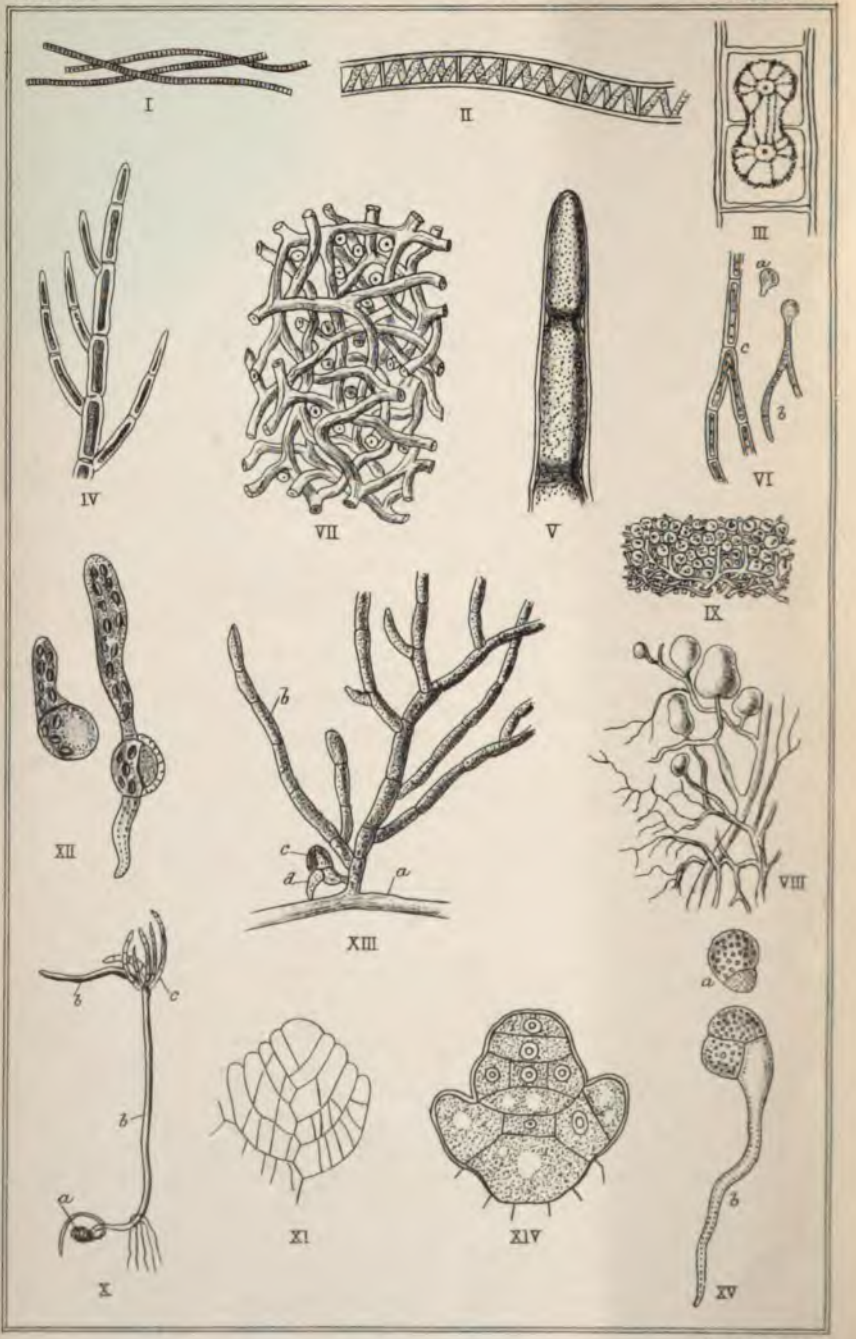
The organism selected to represent the individual life of a green cell is the desmid *Micrasterias denticulata*. It is a not uncommon plant, and may be often found in boggy pools, or in the shallow water ditches of a marsh. The specimens figured and distributed herewith were gathered

appearance. In seasons of less vegetative activity these denticulations may make their appearance, however, before the offshoot has arrived at even half the size it will eventually attain, and most of the multiplying individuals observed in the present gathering were following this latter mode of new half-cell increase.

All the individuals referable to the class to which Micrasterias belongs (the DESMIDIÆ) are inhabitants of fresh water and are characterised (among other points) by the separation of the chlorophyll-bearing protoplasm into two portions within the same cell. In some, division of cell simply takes place by construction or formation of a cell-wall along the median plane of colourless protoplasm, while each new cell so formed grows independently, and attains in time the size and appearance of the original or mother cell. In many cases the cell wall surface grows equally, as may be observed in *Closterium*—a desmid found in our present gathering, and as no pains were taken to exclude it, it will, in the majority of cases, appear in the same "mount" along with its near relation the Micrasterias. In the various species of *Staurastrum*, on the other hand, we have forms whose cell wall surfaces grow unequally as in Micrasterias, producing some of the most beautiful and varied forms of cells to be met with in the whole vegetable kingdom. Outside the desmid's own class we have of course many unicellular green plants, some like *Chlamydococcus*, whose cell-wall surface, growing equally all round, produce a more or less globular cell, while others like some species of *Polyedrium* produce more or less star-shaped cells through unequal growth of the surface of the wall; while in yet other cases we have as in *Coelastrum*, an elongated, club-shaped cell, the endochrome being confined to the upper and wider portions of the cavity, while the free end of the lower elongated hyaline portion is actually attached to some support, thus foreshadowing the development of roots as hold-fast organs in the higher plants.

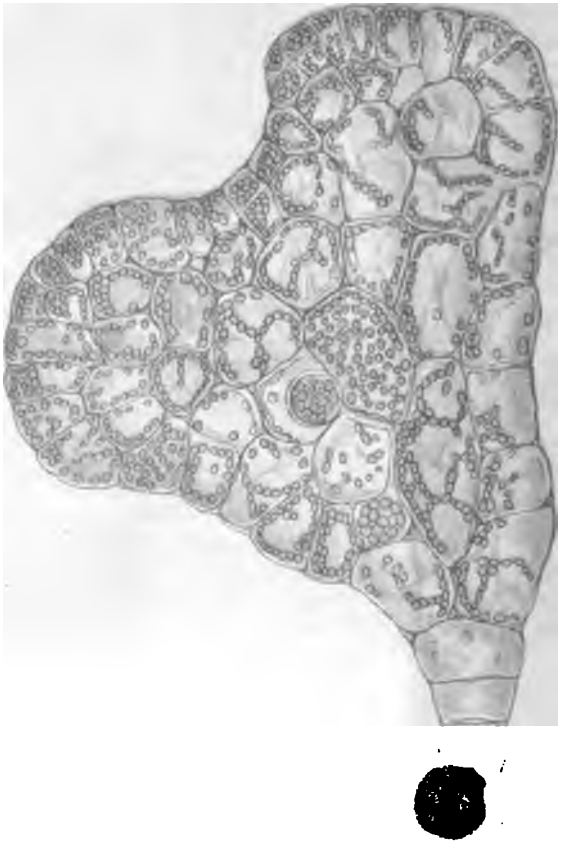
In some of the Desmidieæ (*Didymoprium* and *Sphærozosma*, for example) after division of cell has taken place, and even after the daughter-cells have attained their full development, instead of these newly-formed cells separating from one another, they remain, cohering in the midst of a rather copious supply of gelatinous cell-wall material, the rod-like colony so formed having an appearance strongly resembling a multicellular individual. In the same water as Micrasterias, a few similarly-formed colonies of a species of *Scenedesmus* were observed. These algæ are small, oval-shaped organisms, living by association in rows, the two end-cells being each seemingly provided with a pair of slender, or spine-like horns.

In *Pediastrum* we have still another example of a similar mode of life. Here the cells form a four-, eight-, sixteen-, or thirty-two-celled, flat, disciform colony, the outer boundary cell-wall surfaces of the marginal cells growing unequally result in the formation of two incurved horns to each cell; while in *Gonium*, *Pandorina*, and the well-known *Volvox*, we have this association-ship of unicellular forms carried to the very highest state of morphological development.



TYPES OF SIMPLE TISSUES.

Weekes & Son, Lith. 91, Cr Charles St Berm.y



PROTHALLUS OF FERN

CHAPTER III.

THE MORPHOLOGY OF TISSUES.

Introductory.

If portion of the thin layer of dark green slime so commonly seen covering the sides and bottom of muddy roadside gutters be carefully gathered, and examined under a suitable power, it will be discovered, in all probability, to consist entirely, or almost entirely, of exceedingly fine, yet rigid, short, wavy threads; each separate filament presenting a slender row of disc-like cells, resulting from the formation of frequent transverse partition walls of extreme tenuity. In presence of a sufficiency of moisture the filaments display peculiar oscillatory movements, hence their generic name *Oscillatoria*. Like many of the Desmideæ these organisms may occasionally surround themselves with a rather copious jelly, and form, in suitable habitats, lumps, or balls of greenish slime.

Oscillatoria differs structurally from *Didymoprium* and other Desmids that form rod or thread like colonies in this important respect; in the latter, as we have before seen, the cells, or structural elements of the filaments, are separate individuals, and in no way organically united, whereas in the former the cells are not only physically, but biologically connected, and the stock of available nutriment contained in the cell-sap of any cell is, as it were, the common property of all the cells in the same filament, and may be used for purposes of growth by protoplasm located in any part of the multi-cellular individual.

The passage of nutritive liquids, from cell to cell, in many-celled plants, has hitherto been explained by a reference to the well-known physical process of osmosis; but from the important results of recent researches by Gardiner,[1] it now seems highly probable that the working protoplasm contained within every living cell throughout the entire individual, even in the highest plants, may be looked upon, in reality, as one mass, as every cell-contained moiety of living substance would appear to be in direct union, one with another, by means of very slender threads of protoplasm, which pass through holes of a corresponding fineness, left in the walls of the tissue cells. From the highly suggestive results of this research, it would, therefore, most certainly seem that the dissemination of nutriment from one living cell to another takes place, not by the operation of a mere physical process of diffusion through a thin membrane, but rather by a vital process of uninterrupted transmission.

Other common types of similar, but larger, filamentous forms may be studied in *Mesocarpus*, *Spirogyra*, or *Zygnema*; algæ of extensive distribution in quiet, fresh-water pools throughout the kingdom. Spirogyra

[1] Quart. Journ. Micr. Sci., Oct., 1882; Roy. Soc. Proc., Nov. 11th, 1882; Quart. Journ. Micr. Sci., April, 1883; Roy. Soc. Proc., April 26th, 1883.

with its characteristic spiral arrangement of chlorophyll bands is, of the three, perhaps the best known; and, as it is admirably adapted for observation it may be briefly referred to here.

As seen during its early growth from the mud-buried spore in the spring time, and at a time when the young Spirogyra consists of a row of merely a few cells, there is a manifest functional distinction between the two end cells; one (the basal) is evidently concerned in holding the the germinating thread to the spore which is safely fixed in the mud; while the other (the apical) is free and specially active, adding new cells to the young individual by repeated bipartitions, and, therefore, functionally differentiated to carry on the vegetative growth of the organism. As the plant grows this differentiation is, however, overcome, as, living in waters where there is no possible risk of suffering harmful transportation, and hence deriving no benefit from being permanently fixed, the basal end soon loses its early special function; while, at the same time, each cell—presumedly along the entire length of the filament—becomes empowered to exercise self-multiplication by transverse division of contents.

This binary sub-division of cell takes place rapidly and during the night. The different stages may be clearly studied by suddenly arresting the life of the active protoplasm, which may be easily effected by immersing (sometime after midnight), a wisp of vigorous specimens in a phial of absolute alcohol which will not only kill the plants, but fix, without contraction, their protoplasmic cell contents, or the different stages of new cell-wall formation may be well seen by causing the protoplasmic contents to suddenly contract, by placing the specimens in dilute alcohol, or solution of sugar. The growth of the new partition wall takes place gradually, and is, seemingly, initiated by a centralization of vitality, quickly resulting in the formation of a median peripheral ring of rather dense protoplasm, in which arises the collar of cellulose cell-wall material. This process of special growth continuing, the cell is ultimately sub-divided, while each twin cell, starting an individual development, increases in length, and ultimately reaches the approximate size of the original or mother cell.

In the genus *Conferva*, represented by the green "Silk-weeds" of our coasts, we have multicellular plants structurally resembling the Spirogyra so far as the arrangement of the cells in a single row is concerned; but here—the plants living in constantly agitated waters,—the basal cell exercises its function as a hold-fast organ, during the whole period of growth, while in the majority of cases, perhaps, the apical cell alone is concerned in the function of vegetative increase.

As types of structure somewhat in advance of conferva, any species of the genus *Cladophora* may be very conveniently examined. *Cladophora ruprestris* is marine, its shrubby tufts of dark green filaments being attached to rocks over the entire breadth of the coasted tide-run; while *Cladophora glomerata* is a very common fresh-water species growing from stones or other objects in almost every clear stream or rock-girt spring.

In this genus, the threads, instead of being simply a single file of cells, have spread themselves laterally by the formation of branches. The primary branches arise in each case from the principal row or axis,

by the concentration of individual cell activity beneath a certain spot in the cell wall. Towards this spot the nutritive sap is specially directed, and the protoplasm here being over-fed, as it were, increases unequally in bulk to the rest of the contents, resulting at length in the growth of an off-shoot, which becomes in time shut off from the main cell by the gradual formation of a cell wall very much after the manner described in Spirogyra. Although the cells in Cladophora are filled with nucleated protoplasm, yet it seems that—unlike what takes place in the vast majority of cases in the higher plants—the nuclei are in no way concerned in the process of cell division. The newly-formed lateral cell so produced will however increase in size, and may ultimately sub-divide, forming in time a multicellular branch of limited growth.

In *Penicillium glaucum* the common greenish-grey mould that covers damp bread, old boots, and moist organic substances generally, we have an organism structurally resembling in most points the Cladophora type, although differing from it physiologically very widely indeed. Living a saprophytic life, it feeds like Torula upon already formed constructive material, and hence its cell contents are devoid of chlorophyll; while the filaments creep along or pierce below, the surface of the supporting mass, only throwing up free aërial branches for the production of spores. The hyphæ branch dichotomously, and, as the branches of neighbouring individuals closely interlace a rather dense, somewhat papery mycelium is the result.

In Mushrooms *(Agaricus) Peziza* and other similar fungi we have examples of how such well-defined structures may be built up out of a vast number of interlacing hyphæ, weaving themselves together after a definite plan, but still the ultimate product of a single spore. For example, when the mushroom spore germinates it forms a branching underground mycelium, which, in process of time, throws up—not solitary unbranched threads as in *Penicillium*—but numbers of erect, aërial freely branching, interlacing filaments for the production of its spores, the mature sporocarp being made up of the well-known, definitely-formed structures—the stipes, pileus, and so forth.

In the stipes of the agaric, the hyphæ—as may be seen in the longitudinal section of that organ—run vertically and parallel with one another and so produce an elongated stem, upon the top of which is borne the structure *(pileus)* carrying the inferior, plate-like, closely-set, radiating, spore-producing organs, the *lamellæ*. A section of the pileus, as given in the accompanying preparation, displays the hyphæ running in all directions and freely interlacing, forming the broad, thick, more or less spongy, cap-like mycelium—but a tissue withal that is built up of simple hyphæ resembling that of *Penicillium*.

The thallus of a *Lichen* is structurally composed of similar interlacing fungal hyphae, growing, not in genetic connection, but parasitically upon unicellular or multicellular algæ, which form a green or "gonidial" layer, completely surrounded by the insinuating threads of the domineering fungus. Physiologically, these fungi possess an immense advantage, as they have their hosts, or food producers, [illegible] control,

and are hence able to live under conditions, and affect habitats, totally unsuited to the wants of any of the other members of this food dependent group.

In *Cladophora* (to return to that type), cell multiplication takes place in apical cells. In the main filament or axis, growth may be described as being practically unlimited, while the frequency of bipartition is often very limited indeed, in the apical cells of the lateral branches.

Chara and *Nitella*, slender-stemmed alga-looking gregarious plants, with whorled leaves, found submerged in many fresh water pools and streams, grow as *Cladophora* does, by terminal and lateral apical cells, and they illustrate, in a marked manner, the apparent comparative complexity of structure, arising from a difference in the subsequent individual course of growth of the successive new cells, cut off from the active apical one.

But before the leaf-bearing structure, which ultimately carries the reproductive organs, arises, a distinctly algal like body—the *pro-embryo* is produced. This is an unbranched oscillatoria-or conferva-like thread, with limited apical growth, which grows directly from the spore, and from some special cell of which, somewhere behind the apical one, an erect off-shoot cell arises, which ultimately develops into the ordinary leafy-stemmed individual.

Every fresh cell arising from the bipartition of the apical follows one of two lines of development; it will either lengthen itself in the ordinary or conferva-like fashion producing a cell often of considerable length; or it will remain short, and, retaining the primitive function of self-division, will proceed to break itself up into a ring of sub-cells by the formation of numerous vertical septa. This plate of small cells is known as a *node*, and as the new cells formed from the apical cell follow the one mode or the other alternately, nodes in the stem are always separated by the long one-celled internodes. The nodal cells then throw out offshoots which, like the branches of *Cladophora* not only in their origin but in the limited growth of their apical cell produce the nodal whorl of leaves so characteristic of the *Characeæ*.

In *Funaria*, or other leaf-bearing Moss, the spore so far resembles a uni-cellular alga, as to consist of a single mass of walled protoplasm, containing oil, grains of starch, and chlorophyll. When this spore germinates, it forms a dark-green, branching, uni-cellular, cladophora-like structure, known as the *protonema*, which may in its growth, cover a comparatively large surface, while, at the same time, possessing the power of assimilation, it may have a somewhat extended period of existence.

Each filament of the protonema increases in length by bipartition of an apical cell, the newly-formed transverse walls being always oblique, an important character in the vegetative development of the moss plant. From one or more of the cells a vigorous lateral filament may vertically arise, the apical cell of which dividing very rapidly and with oblique septa, throws back short cells, cut off in such a spiral manner, that they must of necessity intercross in their growth, and hence a structure of many-cell thickness may be formed, resulting in the formation of a stem which, so far as it alone is concerned, may therefore be looked upon as a highly developed or specialised filamentous alga.

The forked Scale-Moss *(Metzgeria furcata)* a gregarious plant very common on stones, rocks, and trees, presents another and different type of structure, being composed of a flat surface of chlorophyll-bearing cells, forming a thin, membranous, and, as the name implies, forked thallus, varying, in size, from a half to three-quarters of an inch in length. This thallus is only one cell deep, excepting along lines or bands carried backwards from the forks—the midribs—where the cells are several in depth. Along the anterior margin of the thallus, and lying within the depression of each of the younger forks, is an active, apical cell, where all new growth, by cell-multiplication, is initiated. Earlier, that is just after germination from the spore, growth was carried on by means of a single, apical cell, but, as the thallus increased in size, similar cells at different points, right and left, eventually arose, and aided in the production of the bifurcating structure, so characteristic of the mature individual.

When viewed from either the upper or lower surface, the apical cell has the form of an isosceles triangle, the base of which is curved and directed forward, and hence always free or exposed. New cells are produced first on one side and then on the other by the formation of oblique walls, alternately parallel with opposite sides of the flat, triangular mother-cell. The daughter-cells so thrown off form on each side, therefore, a plate composed of a series of diverging cells, the inner, end walls of which form a zig-zag median line carried back from the growing point. The cells so produced still retain the power to divide, and they enter at once upon a rather vigorous period of growth. Each daughter-cell is first of all divided by the appearance of an obliquely transverse wall, forming a posterior (smaller) and anterior (larger) cell, the subsequent growth of which respectively is carried out along very different developmental lines.

The posterior cells—all of which are lying, of course, on one side or the other of the before mentioned zig-zag line—increase by the formation of divisional walls, produced in two directions. The first formed cell wall in each of these cells, cuts the cell in a plane, parallel with the surface of the thallus, thus producing two superimposed cells, and as all subsequent bipartitions are made either parallel with or at right angles to the surface of the thallus, a band of tissue—the midrib—of several cells in thickness is the ultimate structural result.

The anterior cell divides only by vertical walls, and as the cells grow rapidly, and also as cell multiplication is rather frequent, a flat, luxuriant tissue of one cell deep is formed on each side, and these, spreading themselves to the right and to the left, around and beyond the growing apical cell, produce in time the characteristic thalloid forks of our type.

In the common horse-tails or *Equisetum*, the individual passes through two distinct life periods or generations—one a simple, vegetative structure, which grows from the spore, producing early, in point of time, the essential organs of sexual reproduction, and the other a spore-bearing

structure, formed of variously differentiated tissues, the result of a more specialised, vegetative growth from the fertilised oosphere.

The unicellular spore is a walled mass of nucleated protoplasm, and contains, like the spores of *Funaria*, distinct grains of chlorophyll. When the spore escapes from the sporangium of the parent, and falls upon damp earth, it almost immediately germinates. Owing to a one-end growth or elongation, the spore first of all assumes a pear-shape form, and then divides, forming two cells of unequal size. The smaller is the narrower, and its contents are colourless and non-granular. It speedily lengthens, forming a comparatively long root-hair. The larger does not lengthen but divides, the division walls being formed parallel with the axis of strongest growth forming two cells lying side by side. These cells containing chorophyll, and hence possessing the power of assimilation are self-supporting; they enlarge, and division soon takes place again, but this time in a direction at right angles to the first, and, as growth continues, so the cells divide, forming in time a flat plate of cellular tissue—the simple prothallus, or individual, representing the sexual generation of the horse-tail. In this stage the structure of the vegetative body of the equisetum advances no further than that found in the simplest of the scale-mosses, and, it is not until the subsequent growth and development of the generation that arises from the fertilised germ cell contained within the archegonium produced upon the under surface of the prothallus, that the different systems of tissues are formed that are so characteristic of this latter stage in the life history of the plant.

In this latter, morphologically higher structure, apical growth is carried on through the activity of a single cell. This cell has the form of a triangular pyramid, having its curved, three-sided base lying in front, and, therefore, free or exposed. A new cell is cut off in regular, intermittent succession by the formation of a wall parallel in turn with each of the three sides of this apical mother cell, and as each succeeding new cell after its appearance lies at a slightly higher level than its just previously formed fellow—owing to the interim growth of the apical cell—the primary daughter cells traced backward from the growing point, form one-third segments of a descending solid spiral. With the subsequent mode of growth of these cells we are not at present concerned, but the ultimate result of their further division, and clearly marked differentiation may be learned by an examination of a transverse section made through any part of the stem a little distance behind the growing point[1].

The prothallus of a Fern has been selected in illustration of a tissue formed merely of a plate of cells, and the genus *Pteris*, in an early stage of growth, has been made the subject of the accompanying drawing.

[1]STUDIES IN MICROSCOPICAL SCIENCE, Vol. I.; see article—The Field Horse-Tail, page 127, and Preparation and Plate of T.S. of Stem.

Taking the particular individual here represented[1], there is, as may be seen, an uniserial thread of three cells length, as the first-formed product of the germination of the still persistent spore. The rudimentary prothallus grew, so far, at all events, by the formation of transverse walls alone, and, therefore, at this stage, it exactly resembled in its mode of vegetative increase a filamentous alga, and the protonema condition in the life history of a moss. Indeed, in one species of *Hymenophyllum* (the filmy ferns) a cladophora-like protonema is produced from the spore, upon which prothalli subsequently appear as offshoots, exactly as the homologous, sexual generation of a moss plant is similarly produced as lateral buds from the moss protonema.

As the development of the prothallus advanced a partition wall arose in the terminal cell of the row, having a direction at right angles to that of the previously formed transverse walls, producing the two cells that are seen lying side by side immediately beyond the first formed series of three cells. Growth continuing, and cell-multiplication taking place by repeated transverse and longitudinal bipartitions; a broad surface of cells such as is here shown is the ultimate structural result. Imbedded in the protoplasm are well-formed grains of chlorophyll, and by the iodine test the presence of already assimilated starch may, in fresh specimens, be also easily discovered in the cells. Long, unicellular, root hairs are produced from certain cells as the result of an unequal growth of the walls. These are functionally concerned in the in-taking of water and soluble earth salts. The rudiments of sexual organs are also shown in the drawing.

As in *Equisetum*, so we find in ferns that the complicated histological structure[2] that eventually grows up from one of the fertilised oogonia of the simple prothallus, is produced through the activity of a single apical cell, the segments thrown back becoming gradually modified to subserve the functional requirements of a highly-developed organism.

EXPLANATION OF PLATE V.

I. Filaments of *Oscillatoria.*

II. Portion of filament of *Spirogyra.*

III. Cell of *Spirogyra*, in the act of division after contraction of the cell-contents by the addition of the solution of sugar; the portion of the cell-wall, already formed, being thus made visible (THOMÉ).

IV. Portion of branching filament of *Cladophora.*

V. Portion of filament of *Cladophora*, showing process of cell-division (CARPENTER).

[1] As the accompanying preparations cannot possibly be all alike, a typical individual—the one sketched—has been selected and described.

[2] STUDIES IN MICROSCOPICAL SCIENCE. Vol. I. See Art.—Bracken Fern, p. 75, and Preparation and Plate of T.S. of Rachis.

VI. *Penicillium.* (*a*) Spore just commencing to germinate. (*b*) Same further advanced. (*c*) Portion of mature hypha.

VII. Interlacing fungus hyphæ.

VIII. Portion of underground hyphæ of *Agaricus*, with young sporocarps (SACHS).

IX. Portion of *lichen* thallus, showing gonidia surrounded by fungal hyphæ.

X. *Chara*, showing growth of pro-embryo from spore (SACHS).

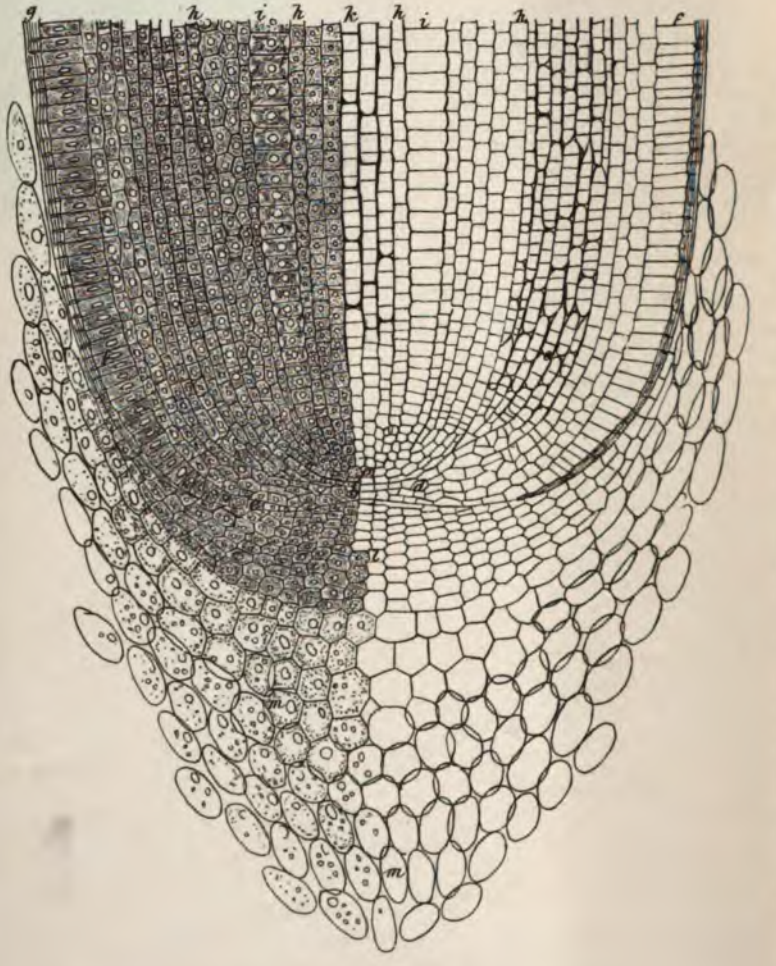
XI. Growing point of *Moss (Funaria)*, (SACHS).

XII. Spores of *Moss* germinating (SACHS).

XIII. *Protonema* of Moss. *(a)* Main filament. *(b)* Branch of protonema. (*c*) Young bud. (*d*) Rootlet (SACHS).

XIV. Growing point of *Chara* (SACHS).

XV. Spores of *Equisetum* germinating. (*a*) Commencement of germination. *b*) Later stage (SACHS).



L.S. THROUGH APEX OF ROOT OF MAIZE

(Sachs)

PRIMARY TISSUE.

In the Vascular Cryptogams as represented by the genus Equisetum, a single apical cell by its growth and continuous bipartition gives rise to a mass of *primary tissue* composed of a number of active daughter cells that are the direct progenitors of all the variously modified, histological elements of the different tissue systems of the mature individual. A similar tissue, having the power of self-division and capable of subsequent development, exists at the apex of the stem and root in plants of a higher order—the Phanerogams. Except, however, in a few extremely rare cases,[1] the primary tissue does not arise in these flowering plants from the activity of a single apical cell, but from several cells (not unfrequently four), all the cells of the group being of equal histological value. The possibility indeed of the existence of a solitary apical cell is, it would seem, entirely prevented by the characteristic mode of cell division that takes place during the early embryological development of these plants.

The oosphere or germ mass in the Phanerogams is contained within the embryo sac—an extraordinarily developed sub-epidermal cell of a special structure, the ovule. After fertilisation is effected, the oosphere clothes itself with a cell wall of cellulose, gets somewhat elongated, and becomes attached, by one end, to the inner wall of the enclosing vesicle. The impregnated oosphere then divides, by a cross-partition wall, into an upper (attached) and lower (free) cell. The upper cell then next divides by the formation of a transverse septum, and, growth continuing, this simple mode of cell increase may be repeated several or many times, producing, at length, either a short or long conferva-like thread known as the *suspensor*. When the lower or *embryo cell* divides, it does so, first of all, by the formation of a median longitudinal wall, hence the two resulting daughter cells are of the same size, and lie, not end to end (as in the suspensor), but side by side, and as they both retain the power of self-multiplication and, also, as the direction of the next division wall is at right angles to the last, a globular mass of four cells is soon found at the end of the suspensor. Each of these cells will again divide, and form an enlarged sphere of eight cells and so on; but it is just somewhere about this stage in the development of the embryo that the first differentiation of tissue manifests itself. Owing to tangential segmentation, an outer layer of cells is cut off—the *dermatogen* or rudimentary epidermis. At this stage, therefore, we have a cluster of cells touched above by the end cell of the suspensor, and bounded on all other sides by these recently developed cells of the dermatogen. This end cell of the suspensor that is in contact with the daughter cells of the embryo-cell, is endowed with a potential of growth and development unpossessed by the other cells of

[1] *Eleocharis palustris*, according to Schwendener, has an apical cell, while on the authority of Dingler the seedlings of *Picea excelsa* have a single apical cell, although this disappears in the older plants.

the suspensor. This cell is known as the *hypophysis*, and is ultimately destined to take a greater or less share in the formation of the embryo. When, in due course, activity sets in, the first formed cell wall lies transversely to the longer axis of the suspensor, and it is the lower of the two cells thus formed that gives rise in a great many cases to the primary tissue of the embryonic root. Just as in the case of the embryo cell so in this the lower cell of the hypophysis, the first formed septum arises in a median longitudinal direction forming two twin cells lying side by side; and this division by longitudinal walls may continue until a number of cells are formed, after which, by the formation of transverse or tangential walls, a layer of outer cells arise, which are continuous with the similar but previously formed dermatogen cells of the rest of the rudimentary plantlet.

Reference to Plate 7.

Longitudinal section through the apex of the Root of Maize (after Sachs).

a. group of apical cells.
b. calyptrogen.
c. dermatogen.
d. periblem.
e. cortex.
f. epidermis.
g. cuticle.
h. i. k. plerome.
  h. cells that will develop into xylem or wood.
  i. row of cells that will produce a vessel.
  k. pith.
l. younger layers of root-cap.
m. older layers of root-cap.

It is in this way, therefore, that the developing embryo becomes possessed of two apical growing regions that tend to lengthen the plant in two opposite directions; and where growth, instead of being carried on by single apical cells, takes place in each case amid a group of cells that arose as we have just seen as the result of longitudinal, instead of transverse, septa, taking place in each of the two primary cells of the future stem and root.

As cell multiplication goes on, and development advances, a further differentiation of tissue soon takes place in the now slowly lengthening organism. The majority of cells pushed back or left behind by the active apical cells, enter upon a special course of individual development, and consequently lose all power of self-multiplication; some for example are destined to become fibrous, others to become fused together in rows

NOTE.—In the plate, one half of the Section is drawn, as it appears, under normal conditions; in the other half, the Section has been subjected to the action of potash which has dissolved out the protoplasm, and left the cell-walls clearly defined.

forming tubes, while the cells in another region may suffer little or very little change in form, becoming simply inactive through loss of protoplasm,—and so on.

A central mass of tissue may be early distinguished, as being the primary tissue out of which the future pith and fibro-vascular systems will arise. It is known as the *plerome*. Surrounding this, and bounded externally by the dermatogen or rudimentary epidermis is the *periblem*, or primary tissue of the cortex. As the radicular end of the embryonic axis grows, at first, much more rapidly than the plumule or stem end, the radicle, in most embryos is much more conspicuous than the plumule, whose longitudinal growth is usually delayed by the production of lateral outgrowths, or rudiments of future foliage leaves. The radicle is, therefore, a more convenient subject than the plumule, for studying the histology of the growing point, and it is the growing point of the root of a germinating embryo of Maize, that has been selected to typically illustrate the primary tissue of Phanerogams.

While the region of apical growth in the stem is protected by the rudimentary leaves of the bud, the growing point of the root (which produces no lateral parts, at all homologous with leaves), is furnished with a special protective development, the root-sheath or root-cap. This, in the Maize, is derived (according to Janczewski), from a special region of active or meristem cells lying in front of the growing point, and to which he has given the name of *calyptrogen*. The new cells cut off by this vigrous layer, are gradually pushed outwards, adding layer after layer to the depth of the "cap," until a structure of considerable thickness is formed. As the cells become further and further removed from the layer of primary mother cells they gradually lose their contained protoplasm, and eventually die; hence an inner part where the cells are filled with protoplasm and closely arranged; and another region where the cells are empty and rather loosely placed may be easily distinguished in the section. The cells of this latter region are being continually rubbed off by friction against the particles of earth in the soil, in which the roots of the plant are growing.

Lying behind the group of apical cells and taking up a central position in the section, we have the plerome. In this, longitudinal rows of short but broad cells completely filled with granular protoplasm, and each exhibiting a very large globular nucleus may be clearly seen, under even a 1-inch obj. power. Associated with these are narrower, but longer, or at least as long, cells, also filled with granular, nucleated protoplasm. Some of these will develop into fibres or wood cells; while others will contribute to the formation of a pith.

The periblem lying around the tip of the plerome (on each side of it in the section) is also made up of small cells completely filled with granular nucleated protoplasm, but if this be traced backwards into the cortex (which as has been already said, is produced from it) the individual life history of each cell may be very clearly traced. As the cells get older, they enlarge

and vacuoles or water cavities soon make their appearance in the midst of the protoplasm. Further back, representing still older cells, the vacuoles have become much bigger, and the nucleus may be generally seen lying in the centre, or almost in the centre of the cell, with strings or bands of protoplasm connecting it with the peripheral protoplasm or the protoplasm closely adhering to the inner side of the wall of the cell. In yet older parts of this tissue the various vacuoles in each cell have coalesced to form one large sap cavity, the nucleus being driven to one side and forced to embed itself in the peripheral protoplasm.

The dermatogen, it seems, is derived from the same meristematic layer as the periblem: continued backwards, it forms the epidermis, the outer or exposed walls of which become somewhat thickened and eventually coalesce to form a continuous, specially protective layer—the cuticle.

In different plants, or groups of plants, there are widely varying differences in the histological structure of the apical meristem region of the root. The most recent contribution to the comparative structure of growing points is comprised in the comprehensive researches of JANCZEWSKI. These have been summarised by Dr. Vines, from whose abstract we extract the following notes:—

Type 1. The meristem consists of four distinct layers, Plerome, Periblem, Dermatogen, and Calyptrogen; *Example*, Hydrocharis Morsus Ranæ.

Type 2. A distinct Plerome and Calyptrogen; the Periblem and Dermatogen have common initial-cells; *Examples*, Many Monocotyledons (Maize, &c.)

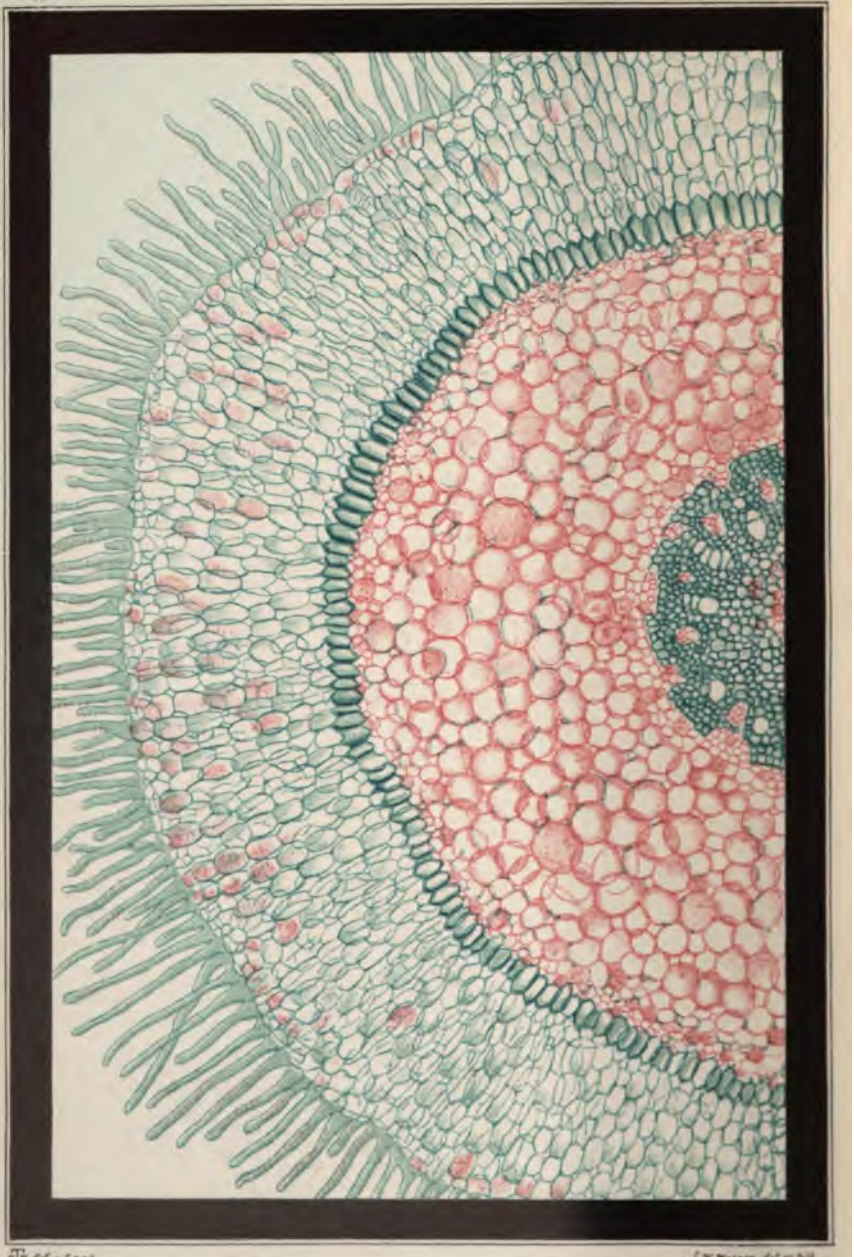
Type 3. A distinct Plerome; the Calyptrogen, Periblem, and Dermatogen have common initial cells; *Examples*, Many Monocotyledons (Lilies, &c.)

Type 4. A distinct Plerome and Periblem; the Dermatogen and Calyptrogen have common initial cells: *Examples*, Most Dicotyledons.

Type 5. A group of initial cells common to all four layers; *Examples*, Some Dicotyledons (Cucurbita, &c.)

Type 6. A distinct Plerome and Periblem only; hence there is no true epidermis or root-cap, these being formed simply by the outer layer of the Periblem (cortex); *Examples*, The Gymnosperms (pines, &c.)

Concerning, therefore, the apical growth of the roots of PHANEROGAMS, the different modes may be thus generally summarised. In GYMNOSPERMS there is neither a true rootcap nor Epidermis, these being simply formed by the outer layer of the periblem. In the two great divisions of ANIGOSPERMS a true rootcap and epidermis are both present. In *Monocotyledons* there is no genetic connection between the Epidermis and the rootcap, this latter structure being derived from a distinct layer —the calyptrogen; while in *Dicotyledons* there is no such special histogen, the rootcap being derived from the dermatogen, and, therefore, in genetic connection with the epidermis.



T. S. OF AERIAL ROOT OF DENDROBIUM.

X 130

EPIDERMAL TISSUE.

In all the higher or vascular plants, we meet with a more or less well-developed, and, genetically distinct outer or boundary tissue-system—the *Epidermis*, which, in its earlier stages at all events, consists of a single layer of flattish, closely-fitting cells, usually sharply differentiated from the underlying tissues. Certain cells of the epidermis may develop into guard cells of stomatæ, the portals of an intercellular aërial system; while others may produce hairs of various forms and functions.

Among the lower forms of vegetable life, as included in the sub-kingdom THALLOPHYTA, there is no absolutely strict morphological distinction between the general mass of body tissue and its sometimes apparently distinct, investing, protective layer. Even in such individuals as *Laminaria* among the Sea-weeds, and Puff-balls among the Fungi, the dense and clearly seen boundary layer of the one, and the easily separable boundary layer of the other, are formed of cells genetically equivalent to those of the rest of the thallus, but physically and functionally modified to subserve the special requirements of their respective growths.

As we ascend, however, into the next sub-kingdom—the MUSCINEÆ, we discover a number of forms, each presenting a truly distinct epidermis. In *Metzgeria*, referred to on page 21, we have a simple member of this small, but highly interesting group: but the thallus of Metzgeria being—with the exception of the "mid-ribs"—merely one cell deep, a boundary layer of any kind is, of course, entirely absent. In *Anthoceros* (Horn-liverwort), although the lobed thallus is formed of several layers of cells, yet the upper or exposed layer presents no differentiation whatever from the layers upon which it rests—in other words there is no real epidermis; while in *Riccia* (Crystalworts), on the other hand, a clearly-defined epidermis, but without stomatæ, is always present upon the upper surface of their flat, often deeply-forked, thalloid stems. In the *Marchantieæ* (Marchantia, Lunularia, Fegatella, &c.) the thallus not only presents upon its upper surface a very distinct epidermis, but an epidermis provided with stomatæ, the guard cells of which, in many cases, being more than usually complex, owing to the repeated bipartition of the original mother cell of the stoma-bounding group.

In the true Mosses *(Musci)*—but excepting the Sphagnums—the leafy plant of the first or sexual generation has, as a rule, no properly

differentiated epidermis, the outer or boundary layer, or layers, of cells (in the stem) being merely smaller, thicker-walled, and more closely packed than the inner or axial cells. It is not until the moss plant enters upon its second stage, or spore-producing life period, that a distinct epidermis (furnished by the way with peculiar stomatæ), makes its appearance, and then only upon the developing spore fruit.

In *Sphagnum* (Bog-mosses) the stem is provided with a very conspicuous epidermal tissue-system, composed, in some species, of only a single layer of cells, but, in others of two or four such layers. The cells are large, colourless, and thin walled and contain either air or water. The frail walls are generally strengthened by slender, spirally arranged thread-like thickenings, while in many cases the cells are in direct communication with one another, and with the outside by means of minute pores. It is up this tissue, as through a sponge, that the water rapidly passes, and within this tissue, also sponge-like, that water is retained by these bog-loving plants.

In the two groups lying above the mosses—that is, in the VASCULAR CRYPTOGAMS and PHANEROGAMS—an epidermis is an invariably occurring structure, clothing alike the roots, stem, and leaves, and becoming variously modified under the influence of individual conditions of growth, and by the physiologically imperative requirements of the plant. In its origin the epidermis of Phanerogams arises, as we have before seen, from the dermatogen, a structure that makes its appearance at an early stage in the plant's embryonic development. As it gets older, the cells become more or less flat, while whatever changes may take place in their outer or exposed walls, the radial walls always remain thin and possibly porous. Simultaneously with these changes certain developments may take place among its cells, some, as has been before stated, may produce hairs, while others may become the guard cells of stomatæ. Hairs are freely thrown out by the epidermal cells of the roots, and these, under many circumstances, are functionally concerned in the absorption of water for the benefit of the whole plant. Hairs are also found upon the epidermis of stem, leaf, and flower, and are utilised for the performance of the most varied functions; ordinary hairs seem to be especially produced to decrease the rate of transpiration, hence exposure to air and light tends to promote an increased development of these appendages. Sometimes a hair is a stinging organ, as in Nettle; a digesting organ, as in Sundews; a scandorial organ, as in Bramble; and so on.

When stomatæ make their appearance upon the epidermis, they are invariably situated above intercellular spaces, to which they offer a direct means of communication with the outer air. They are not formed in subterranean or subaqueous structures, and their formation seems to be regulated (in addition to heredity) by retardation of growth, and an available accumulation of food material: as has been partially proved by growing in the air branches of certain water plants that naturally grow entirely submerged. It was found that, concurrently with a slower growth, and greater concentration of food, a number of stomatæ made their

appearance upon the artificial ærial leaves. While further, it has been recorded that stomatæ have been found on galls upon the upper side of the leaf in vine, although normally they only occur upon their lower surface. Here the accumulation of food, due to the flow of sap to the seat of irritation, was evidently the chief factor in the formation of these, in this case, apparently functionless stomatæ.

When the epidermis is fully formed, and, if it is subject to aërial exposure, the outer boundary walls of the cells are often considerably thickened, sometimes enormously so, as in many leathery leaves like holly, mistletoe, and the like.

There exists upon the surface of most leaves a continuous, membranous, transparent layer, the *cuticle* possessing most extraordinary resisting power, and useful not only for purposes of protection, but as a check to transpiration, and thus preventing a too rapid loss of water.

Iodine colours the cuticle brown, while oxidising agents and boiling potash water dissolves it. A resinous or waxy substance, soluble in boiling alcohol, covers the surface of the cuticle and forms an effectual protection from the wet.

It would seem that in addition to the function of protection, the epidermal cells perform other and important duties in the economy of the plant. They store up a reserve of water which, owing to the thinness of their radial walls can easily pass from cell to cell, thus enabling them to meet within certain limits any extraordinary demands brought about by the necessities of transpiration, while at the same time they are also able, should the necessity arise, to deliver up to the chlorophyll-bearing cell a quantity of water for purposes of assimilation.

Occasionally the epidermis gives rise, as in the leaves of Ficus, Begonia, and many Piperaceæ, to an inner layer of cells with thin walls and watery contents. They are functionally concerned in the storage of water, and are known as *aqueous* cells.

In the aërial roots of Orchids and Aroids there is, outside the true epidermis an extraordinary cellular development that completely invests the root stretching from the extreme tip to its point of insertion in the stem. This structure has a remarkable power of absorbing water, and is known as the *Velamen.*

A transverse section of the aërial root of a species of Dendrobium, double stained, and well exhibiting this velamen growth, has been selected as the subject of study under our present heading. Its structure, together with the structure of the epidermis proper, may be readily made out from the accompanying preparation, by working through the following instructions. It may be explained that although we are at present mainly, if not wholly, concerned with epidermal tissue, yet I have not hesitated to point out the most important features to be observed in other parts of the section.

It will be well, first of all to make out the following naked-eye characters in the section :—

(*a.*)—An outer system (the *velamen*) stained a palish green, having its free surface clothed with a number of closely-set hairs of various lengths.

(*b.*)—A broad ring of tissue (the *cortical* system) lying within (*a*). It has taken up the red stain.

(*c.*)—A thin, dark, circular line (the true *epidermis*) lying between (*a*) and (*b*).

(*d.*)—A central disk of dark-green stained tissue (the *axial fibro-vascular bundle*) surrounding a mere speck of clear looking tissue (the *pith*).

With the help of an ordinary pocket lens make out further :—

(*a.*)—The clear-looking, thin-walled oblong cells of the *velamen*, so disposed that their longer axes lie radially in the section.

(*b.*)—The thick-walled, dark-green stained cells of the *epidermis*.

(*c.*)—The roundish, thin-walled cells of the cortex.

(*d.*)—The concentric arrangement of alternate large (elongated), and small (roundish and red-tinted) patches, composed of the cut ends of more or less wide tubes ; the larger, representing the vessels of the wood, and the smaller the equivalent structure of the bast.

(*e.*)—The round cells of the pith.

Next examine with an 1 inch obj., and observe :—

(*a.*)—The delicate, intercrossing, spiral thickenings upon the walls of the *velamen* cells ; the origin of the hairs, each being, simply, a prolongation of the exposed or free wall of one of the outer or boundary cells of the *velamen*.

(*b.*)—The circle of distinctly differentiated cells of the *epidermis* ; carefully noting, however, that the continuity of the thick-walled laterally compressed angular cells, that make up the greater portion of the layer, are here and there interrupted by the intercalation of solitary, more or less, roundish parenchymatous cells with slightly thinner walls, but resembling the other cells of the tissue-system so far as the selective absorption of the green dye is concerned.

(*c.*)—An outer ring of small, angular, cortical cells lying against (*b*) ; a single layer of inner cortical cells (the *bundle* or *plerome sheath*) surrounding the axial fibro-vascular bundle : the file of cells lying opposite each region of bast, having their walls considerably thickened, and consequently green stained ;—the remaining and larger cortical cells presenting blue-tinted, irregularly banded thickenings, more or less distinct, especially if the section is a little thick.

(*d.*)—(1). A distinct outer layer (the *peri-cambium*) lying immediately within the plerome sheath, and, like that layer, having the walls of the cells thickened that lie opposite the region of bast.

(2). The bast or phlöem composed of a central twig of cells and vessels (*soft bast*) coloured red, surrounded by thick walled cells (*bast fibres*).

(3). The wood or xylem, composed of *vessels* with very wide cavities, to the outside of which is a region of narrow cells (*wood prosenchyma*) extending to the layer of pericambium, the cell walls in all are stained green.

(*e.*)—The pith composed of roundish cells occupying the centre of the plerome.

Finally examine with the quarter inch objective noting particularly under the increased magnification.

(*a.*)—The spinal thickening upon the walls of the cortical cells.

(*b.*) The walls of the epidermal cells,—the outer and lateral walls thick, the inner walls comparatively thin.

(*c.*)—The cut faces of short breadths of green-stained thickening bars, upon the wall of the round cortical cells, the thickenings on contiguous walls being opposite to one another, and so disposed, that they are generally seen upon the segments of weakest pressure (that is, bordering intercellular spaces) the faces of contact with neighbouring cells remaining thin walled, thus permitting an easier communication between cell and cell. Faint outlines of feebly-stained bands may be made out within many or most of the cells.

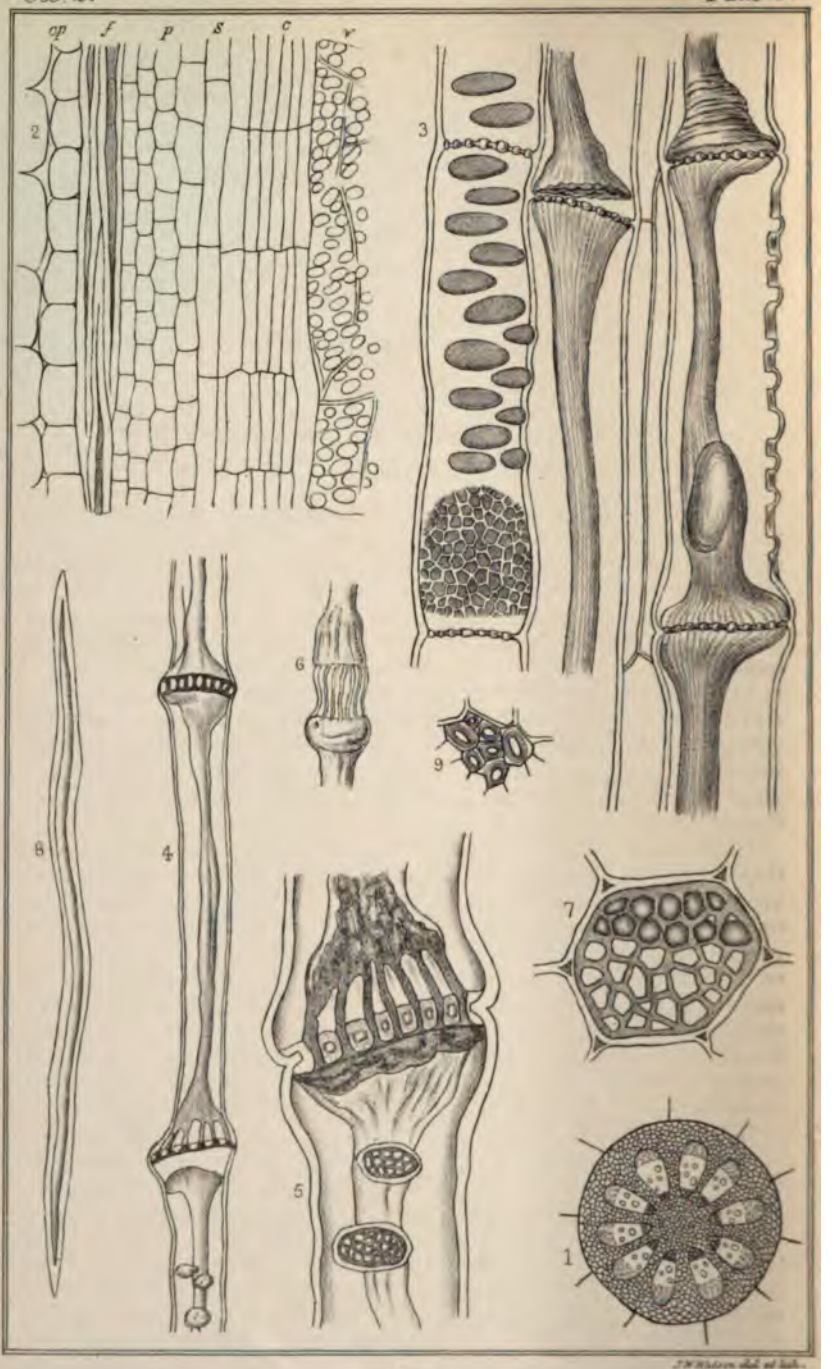
(*d.*)—The thick walls of the bast cells.

With respect to the further study of the epidermis and its special covering the velamen, recourse must be had to longitudinal sections, or better still, perhaps, to maceration and subsequent teasing and scraping, or if a fragment of the root be boiled for a few minutes in water, to which are added a few drops of caustic potash, the cohesion of the cells will be sufficiently overcome to render examination tolerably easy. If the velamen cells be carefully scraped away from above, and the cortical cells from below a portion of the epidermis, a thin, tough, transparent tissue will be isolated, the structure of which may be easily made out with a moderate power of the microscope. The cells, it will be seen, are much elongated, having straight parallel side walls, and depressed or concave ends. As the cells are disposed in rows, a more or less circular opening is left between the uniting ends of any two cells in every row, and lying within each of these spaces is a sub-globular cell with thickened walls, and pierced with many pores.

If the fragment of root be boiled in the alkaline water for a lengthened period, the long cells will readily separate, and, closely set, transverse thickening bars upon the side walls may be easily made out under a proper adjustment of the light. Some of the small cells belonging to the outer region of the cortex will be almost sure to be adhering to some parts of the epidermis under examination: these will be seen to be oval-shaped, with thickened walls, plentifully dotted with either thin places, or actual openings, and if a further examination be made

rest of the cortical cells, all gradation from these cells to larger ones, where the thickenings are disposed in irregularly disposed bands of varying thicknesses (reminding one of the parenchymatous cells from the lower portion of the thallus of Marchantia)—will be readily observable.

Having completed our observations of the minute anatomy of these remarkable aërial roots of Dendrobium; and if we now remember that this genus of orchidaceous plants grow in such situations (that is, upon trees), where ordinary roots can do little more than merely play the part of holdfasts: and, if, at the same time, we remember that the plants affect regions where the warm air is for ever loaded with an abundance of watery vapour, we can, I think, readily understand how functionally serviceable such aërial root organs as these must prove themselves to be in the physiological economy of this or similar epiphytal organisms. Springing from the exposed base of the succulent stem, the green-tipped roots soon become curved, and eventually freely hang in longer or shorter cords in the moisture-bearing air. Water, be it rain or dew, is, strictly speaking, scarcely pure, containing as it does dissolved ammonia and various salts derived from the impurities of the air; and this water, possessing a varying nutritive value is readily absorbed by the thin-walled velamen cells until that tissue becomes more or less gorged. The true epidermis has evidently a merely skeletal function, a cylinder of strength, as it were, between the two regions of mechanical weakness—the velamen and cortex. Through the very thick walls of the cells of this epidermal system the water would find a slow and difficult passage, but, as we have just seen, the continuity of these cells is interrupted by the presence of numerous small, isolated cells provided with pores, and through these the water from the turgid velamen cells readily and directly passes into the banded cells of the cortex; while from these cortical cells the axial fibro-vascular bundle obtains its supply of water, and this very essential fluid, the bundle, in pursuance of its ordinary duty, will speedily carry upwards into the stem.



BAST.

Sieve Tubes & Liber Cells.

VASCICULAR TISSUE.

1.—General Structure of a Fibro-Vascular Bundle.

A distinctly differentiated tissue-system usually assuming the form of isolated cords or strands, is commonly found traversing the various organs of all the higher or leaf-bearing plants lying above the Mosses in the scale of complexity of organisation. In this group (that is the true mosses) there appears for the first time an axial string of dense or closely packed cells, considerably elongated in the direction of the plant's most rapid growth, and still further differing from the other cells of the stem in having their walls regularly thickened to an appreciable extent. Such a system as this, composed merely of simple fibres, obviously foreshadows the much more complex, but still exactly equivalent system of fibres, tubes (or vessels), and cells that make up the histological elements of the ramifying cords (or fibro-vascular bundles) of the Vascular Cryptogams, Gymnosperms, and Phanerogams.

In our study of the growing point of Maize, it was therein learned that during the whole period of its embryonic condition, a plant is made up entirely of cells ; and that, although at one stage, these cells are to all intents and purposes exactly similar, so far at least, as outward form or general appearance goes, yet the individual cells of different groups are so fully endowed by the strong force of heredity, each with its own special physiological potential, that different cell-groups are thereby compelled to develop towards a permanent or mature state, along certain lines in gradual yet definite stages of growth, resulting at length in the production of all the different systems of tissue that are so markedly characteristic of the species to which the individual in question belongs.

Referring to plate 7 we see, for example, in the longitudinal row of short broad cells marked (*i.*) an early stage in the development of a vessel. Subsequently in the life of such a row, the superimposed cells would become completely fused, and the transverse separating walls become, perhaps, first ruptured, and then probably absorbed while simultaneously with this the contained protoplasm would gradually thicken certain parts of the now long and common wall, by the addition of new cell wall material, elaborated in the form of bands, spirals, or the like. Such special tubular structures are clearly of great functional importance, inasmuch as by virtue of the peculiar mode of thickening, not only is there a considerable gain of strength, but the thin or unthickened portions of the vessel-wall permit, at the same time, an easy passage of gases in a lateral direction.

A fibro-vascular bundle is made up of two distinct portions—the xylem or wood, and the phloëm or bast; but while differing considerably from one another, both histologically and functionally, each region is nevertheless typically made up of fibres, vessels and parenchymatous cells. The vessels, however, present the chief differences, as, while in the wood these structures, as we have just seen, are completely open or continuous throughout, and secondary thickenings are freely deposited upon the side walls; in the vessels of the Bast, the elements retain to a greater extent their cell individuality, owing to the non-destruction of their transverse partitions; at the same time the walls show little tendency to become thickened, while numbers of minute pores are found upon either the end or side walls permitting direct inter-communication between the protoplasmic contents of the entire "vessel."

In all the vascular cryptogams, excepting the *Equisetaceæ*, the bundles are *concentric*, that is the xylem is entirely surrounded by bast; while in the Equisetums and in most Phanerogams the xylem and bast are radially disposed with respect to one another, the xylem being towards the centre and the bast towards the circumference as in Fig. I. in the accompanying plate. Such bundles are known as *collateral*.

Collateral bundles may be *open*, as in typical Dicotyledons, where a region of cambium separates the woody from the bast portion; or the bundles may be *closed*, as in Monocotyledons, where no such generative layer exists. In a few Dicotyledons two regions of bast are present, one in the usual position, or on the circumferential side of the wood, and another on the inner or axial side. These have been called *bicollateral* bundles. In the subject of our last study (the aërial root of an orchis), it was incidentally observed that the xylem and bast of the axial bundle were therein arranged alternately, that is, in different radii; this arrangement is (with very few exceptions) universally characteristic of roots, and such bundles are known as *radial* ones.

In the subject of our present study—a preparation of the transverse section of the stem of *Cucumis*—it will be found that the elements of the bundles are arranged in a bicollateral manner, and hence in this respect the cucumber is an exception among the rest of its class. With the view of obtaining a general notion of the structure of a fibro-vascular bundle and its position and comparative histology with respect to the other tissue elements of the stem, the accompanying preparation may now be examined, according to instructions given below, and sketches made of the various systems and groups of tissue as they appear under the different magnifying powers used.

The following characters may be made out with the simple lens:—

(*a.*)—The somewhat irregularly waved outline of section; the lobes agreeing with the distinct ridges that run parallel with the direction of growth in the stem; the hollows with the corresponding grooves or channels.

(*b.*)—The *epidermis* consisting of a narrow line, just slightly darker than the immediately underlying tissue.

(*c.*)—The *hypoderma*, a dark band of closely packed cells, separated from the epidermis in the ridges by a depth of thin-walled parenchymatous cells, but actually touching it in the channels.

(*d.*)—The *fibro-vascular bundles* arranged in two groups or systems. An outer sub-circle corresponding with the ridges, and an inner, corresponding with the channels. Each is seen to be made up of (1) a middle region—the *xylem* or *wood*, composed of empty tubes (two at least of which are exceedingly large), intermixed with and radially surrounded by groups and masses of narrow-celled and denser tissue; and (2) end regions (peripheral and central)—the phloëm or bast—of narrow tubes, many of which apparently contain some homogeneous contracted material, and presenting a dotted appearance in consequence.

Examine the section again, using an 1 inch obj. power. Make out:—

(*b.*)—The *epidermis*, a single row of small, but otherwise not very distinctly marked cells.

(*c.*)—The *hypoderma*, of narrow cells, with thickened walls.

(*d.*)—The *fibro-vascular bundle* (1) the thick-walled narrow fibres, and the thin-walled, small parenchymatous cells, together with the large and small vessels of the *xylem*. (2) The vessels of the *bast*, some with contracted contents; others presenting a circular plate continuous with the inner walls of their respective tubes; intermixed with these vessels are narrow, thin-walled, parenchymatous cells; while true bast fibres appear to be entirely absent.

Examine bast region under a higher power—say a $\frac{1}{4}$ or an $\frac{1}{8}$ obj.—and carefully observe the structure of the circular plates noticed in the vessels under the lower power: they are thickly pierced by minute pores, and present a characteristic appearance, hence their name (*sieve-plates*).

Oblique and longitudinal sections of the stem show, that the very wide tubes of the xylem are dotted vessels, the walls of which are additionally strengthened by banded, irregularly-meshed, thickening layers; while the smaller tubes are seen to be spiral vessels.

2.—Bast (Phloëm)—Sieve Tubes and Liber Cells.

The bast, or phloëm portion of a fibro-vascular bundle is, as we have before seen, typically made up of vessels, thick-walled, elongated cells, and succulent parenchymatous cells. In form, the elements of the vessels are usually prismatic, with either slightly oblique, or truncate ends. The walls are invariably composed of pure or unmodified cellulose, and are never thickened to any considerable extent. Certain of the walls (the transverse ones in cucumber) are further characterised by the possession of pores, through which pass connecting threads of protoplasm (Fig. 5)[1]. A peculiar albuminous thickening, known as the *callus*, covers the surface of the sieve plate during the active period of the "tube." This is also shown in Fig. 5. A parietal layer of proto-

[1]The continuity of the protoplasm can be well demonstrated by following the directions given by Sachs. "It is sufficient," he says, "to saturate thin longitudinal sections of the phloëm with iodine-solution until the contents of the sieve-tubes begin to turn brown, and then to add concentrated sulphuric acid; this dissolves the cell-walls and the substance of the sieve-plates, and nothing is left but the mucilaginous contents coloured a deep brown." (See Fig. 6).

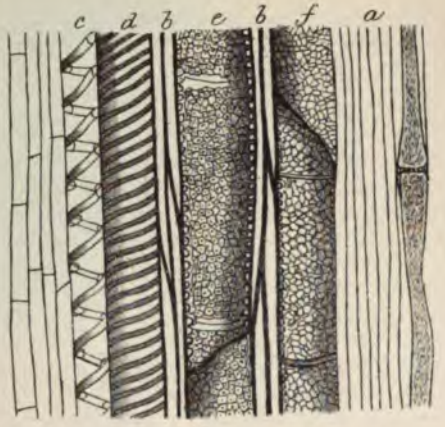
plasm will persist for a longer or shorter period. With its disappearance all activity will cease, and the tube become passive or dead. Although a nucleus is not ordinarily observable, yet in the Scotch fir (*Pinus Sylvestris*), according to Russow, several nuclei exist, when the elements are in a young stage. In addition to the protoplasm, sieve-tubes also contain a watery fluid, a sort of peculiar mucilage and starch grains.

In Dicotyledons, where the bast is developed from the cambium, when a cell is cut off that is destined to take part in the formation of a tube, it first divides longitudinally, and while one of the twin daughter cells evolves into a tube element without further division, the other becomes the mother cell of several bast parenchymatous cells. The row of cells marked (s) in Fig. 2 are young sieve-tube elements. According to Janczewski, a number of symmetrical dots or "warts" of callus substance make their appearance upon the transverse walls, the wart material spreads, until, by the coalescence of the approaching borders, it cover the whole surface of the plate, after which the pores are formed at points agreeing with the position of the warts.

The complete life history of a sieve-tube may be divided into four periods—(*a*) the evolutive, during which the vessel is acquiring its distinctive characters; (*b*) the active, within which it attains its fullest vigour—it contains protoplasm, mucilage, and starch, and performs very important physiological work; (*c*) transitional, during which period it loses its protoplasmic contents, while at the same time the callus begins to disappear also; (*d*) passive, when the tube is empty, and the plate, viewed from above, has merely a recticulated appearance (as may be seen in the lower part of Fig. 7). In Dicotyledons, where, owing to the activity of the cambium new sieve-tube elements are periodically cut off, the changes just indicated may take place in a few months; but in the closed bundles of the Monocotyledons, on the other hand, where the sieve-tubes are formed from the procambium, the activity of the elements may continue for years—indeed, as long as the life of the bundle itself.

In the Vascular Cryptogams, the sieve-tube elements are comparatively small, being, in fact, no larger than the neighbouring parenchymatous cells. It appears, so far as present research goes, that the "pores" are not actual openings but simply pits, and that they occur upon both lateral and terminal walls. In Gymnosperms, as represented by the Scotch fir, the elements are square or longer tangentially. The radial and end walls present irregular thickenings, and lying within these, upon the weaker walls, are the sieve pores, here however actual openings. The Monocotyledons have the pores upon the lateral walls only of the tubes, while the Dicotyledons may have them upon either of the walls—lateral or terminal.

Respecting the cells of the bast, little requires to be said—In Fig. 8, the elongated, thick-walled, pliable liber cell is shown, while Fig. 9, which represents the cut ends of a group of such fibres, displays the characteristic stratification of the thickening layers. In Fig. 2 they are shown at (*f*). The "middle lamellæ" may in some cases be lignified, while in other cases it may be mucilaginous. We have already seen that a system of thick-walled liber cells or hard bast is absent in our type-plant—the cucumber.



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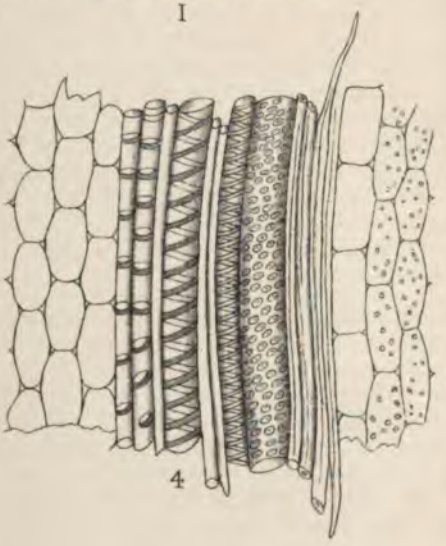

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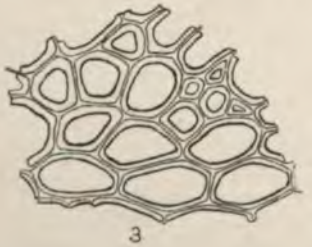

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J. W. Watson del. et lith.

WOOD

Wood Vessels and Cells

Watson & Son, Lith. 31 & 32 Charles St. Birm.

3.—Wood (Xylem). Wood Vessels and Cells.

The Wood or Xylem of a fibro-vascular bundle, is, like the bast portion, made up of vessels, thick-walled elongated cells, and cells of a parenchymatous nature. The vessels form long and, usually, continuous tubes, developed, as hereinbefore described, from rows of superimposed and histologically distinct cells, containing nucleated protoplasm, and bounded by excessively thin walls formed of pure or unmodified cellulose. In the ordinary course of development the transverse partitions eventually disappear, while the lateral walls of the completely fused cellular elements are, in part, thickened by the deposition of new cell wall substance in the form of spirals, bars, hoops, reticulations, and the like. These secondary deposits are gradually built up as the growing point lengthens and new cells are added to the forward extremity of the primary vessel-row. The markings first make their appearance at the lower end, that is, the end further removed from the apex of the axis, as is shown in Fig. 2 (plate 10), which represents a portion of the margin of a fibro-vascular bundle of the common bracken fern, seen in longitudinal section at a spot where, it will be noticed, the parietal thickenings are just making their appearance at the distal end of one of the younger elements of a spiral vessel. Simultaneously with these structural changes, other changes of a distinctly chemical nature arise within the substance of the cellulose cell wall, that give, in this relation, a very distinct character to the xylem or wood vessels.

While pure cellulose is easily dissolved by concentrated sulphuric acid, or by an ammoniacal solution of copper oxide, the material forming the solid portions of the woody tissue remains entirely unaffected when subjected to the influence of these chemical re-agents. This cellulose modification—known under the name of Vasculose—may, however, be readily dissolved by the application of any oxidising substance, such as nitric acid, chlorine water, or permanganate of potash. Vasculose differs chemically from true cellulose in containing more carbon and less hydrogen and oxygen than that well-known carbo-hydrate.

This chemical metamorphosis of cellulose into vasculose is, however, an important one, and has a direct influence upon the physical character of the bundle, inasmuch as the formation of vasculose gives considerable additional rigidity and strength, or power of resistance, to the stem or other organ through which the vascular bundles ramify.

A typical group of vessels is shown in Fig. 4, wherein it is seen that vessels in the same bundle not only differ in appearance, but also in size, and that they are intermixed with elongated thick-walled cells—the prosenchyma. One of the vessels—the largest—is dotted, the "dots," of course, representing the thin, or unthickened places on the cell walls. Two have spiral markings, while two others—the narrowest—may be described as "annular," the secondary deposits having here taken the form of rings.

The preparation sent out herewith—a longitudinal section (double stained)—of the stem of a Sunflower (*Helianthus annuus*), typically and clearly displays the minute structure of the xylem of a fibro-vascular bundle. (See Fig. 1).

In the cucumber—the subject of our last study—it will be remembered that the fibro-vascular bundles were absolutely destitute of true bast fibres, while in the subject of our present study the hard bast (as this region is commonly called) has, on the contrary, attained an almost extraordinary development.

We will find it therefore instructive to examine in the accompanying section not only the xylem portion of the bundle (with which we are at present more particularly concerned), but also the region of the bast, and especially the hard bast; while, at the same time, it will give us an opportunity of observing all the various tissue elements of a fully developed stem, as displayed in longitudinal section. The student will further find it extremely interesting to compare this vertical section with that of the growing point of the root of Maize, described in the present chapter, under the heading "Primary Tissue." In this latter preparation the epidermis, cortex, fibro-vascular system and pith are shown in their primitive or rudimentary state, while the preparation about to be examined displays these systems of tissue in their fully developed or mature condition. It will, of course, be understood that the layer of cambium is a region of persistent meristematic cells, or cells that have not yet passed over into a permanent or specialised form; that each cell in this layer also possesses the primitive power of multiplying itself by bipartition, and that from the new cells generated in this way fresh layers of bast are evolved on the one side, and additional layers of wood or xylem elements on the other.

The following points of structure will be readily made out in the section. It will be well to examine the preparation first under an inch obj. power, and then under a "quarter" or an "eighth":—

(*a.*)—The *epidermis* (slightly green-stained) with scattered uniserial hairs seated upon multicellular sockets.

(*b.*)—The *cortex* (stained carmine), the parenchymatous cells of which are thin-walled, and of different shapes and sizes, but all are more or less elongated in the direction of growth of the stem.

(*c.*)—The *hard bast* (green-stained) very strongly developed, and made up of comparatively wide and moderately thick-walled cells, with obliquely pointed ends, exactly fitting into one another, and forming a dense tissue of closely-set cells.

(*d.*)—The *soft bast* (stained carmine), composed of sieve-tubes with thin walls, and contracted granular contents, together with much elongated parenchymatous cells.

(*e.*)—The *cambium* cells (which have also taken up the carmine stain), lying on the inner side of the soft bast, and made up of

long but very narrow and extremely thin-walled cells, filled with protoplasm; the cells lie in radial rows, while the rows are arranged longitudinally in tiers.

(*f.*)—The *xylem* or *wood* (stained green) made up of wide dotted vessels towards the cambium and narrower spiral vessels towards the inner side: intermixed with these are seen long, narrow, thick-walled tapering cells *(prosenchyma).*

(*g.*)—The *pith* (stained carmine) formed of irregularly shaped cells, varying very much in size and apparently empty.

Respecting the vessels noted above under (*f*) it should be observed that the transverse partitions indicating the boundary between superimposed cells may be very clearly seen in all the vessels displayed in the section. In this case the end walls have only suffered partial absortion, and are interesting as being forms of vessel somewhat transitional between simple cell fusions and wood vessels proper. They also demonstrate (on being compared with similar primary tissue cells out of which the vasicular system was evolved) that during the formation of the first-formed fibro-vascular bundle all the cellular elements were lengthened to a considerable extent, and further, that as the first formed vessels arise towards the pith, and as therefore the parietal thickenings would be deposited therein before such vessels had attained their full length, the spirals have, to a certain degree, been pulled out under the influence exerted at that time by the stretching walls of the then growing vessels.

A transverse section of the Sunflower (and double stained) should either be made or procured by the student, and compared, first, with the longitudinal section of the same plant, and, second, with the preparation of a transverse section of Pinus Sylvestris, previously distributed with the present series of "Studies" (slide No. 2, plate 2).

In a transverse section of the stem of the Sunflower each fibro-vascular bundle is seen to be made up of a large patch of hard bast (often sub-circular in form), to the inner side of which lies a much smaller region of soft bast, with its convex outline lying against the narrow band of brick-shaped cells of the cambium. The xylem displays towards its outer side a closely grouped assemblage of prosenchymatous cells (Fig. 3), and vessels presenting a concave surface towards the cambium, and sending rays of wide-tubed vessels among the parenchymatous wood cells that form the principal constituents of the bundle towards its inner side.

In the transverse section of the young shoot of *Pinus* (and it would be well to examine also a longitudinal section of the same), it will be observed that with the exception of a narrow region of spiral vessels bordering upon the pith, all the rest of the Xylem portion of the bundle is made of prosenchymatous cells, which, in longitudinal section display "bordered pits" upon their lateral walls (Fig. 5, plate 10). When the thickening process sets in, in these cells, comparatively large circular areas are left at different points on the walls, but invariably at spots opposite to one another, in neighbouring opposed cells. As the thick-

ening proceeds, these areas are in time spanned by an all but closed in dome roof, owing to the pushing over, as it were, of the thickening material, until when growth ceases, a small circular opening only is left facing the interior of the cell. The thin double wall of each unthickened area is eventually absorbed, and direct communication is thus established between all the cells of the bundle (Fig. 6).

EXPLANATION OF PLATE 9.

Fig 1. T. S. of the stem of Melon, showing the concentric arrangement of the collateral fibro-vascular bundles (mag.) *(Le Maout and Decaisne).*

„ 2. L. S. of portion of a fibro-vascular bundle of *Ricinus*, showing *(c)* cambium, *(s)* row of cells that will afterwards develop into a sieve-tube, *(p)* bast-parenchyma, *(f)* bast-fibres, *(c.p.)* cortical parenchyma, *(r)* portion of vessel of the wood *(Sachs).*

„ 3. L. S. of phloëm of *Cucurbita Fepo*, showing two young tubes (with contracted contents), and a single tube (to the left), with sieve-plates in course of formation *(Sachs).*

„ 4. Sieve-tube of *Bryonia dioica* in longitudinal section *(Thomé).*

„ 5. L. S. through a transverse partition-wall of the gourd, showing the callus and the connecting threads of protoplasm *(Thomé).*

„ 6. Preparation from a sieve tube of cucurbita after solution of the cell wall with sulphuric acid *(Sachs).*

„ 7. Sieve-plate viewed from above; upper part represented as being in an active condition; lower portion as being in the passive state *(Thomé).*

„ 8. A thick-walled bast cell (fibre).

„ 9. T. S. of a group of bast fibres.

EXPLANATION OF PLATE 10.

Fig. 1. Radial longitudinal section of stem of Sunflower *(a)* cambium [with a sieve-tube to the right], *(b)* wood-fibres, *(c)* small, and *(d)* large spiral vessel, *(e)* pitted vessel, *(f)* pitted vessel in course of formation. *After* PRANTL.

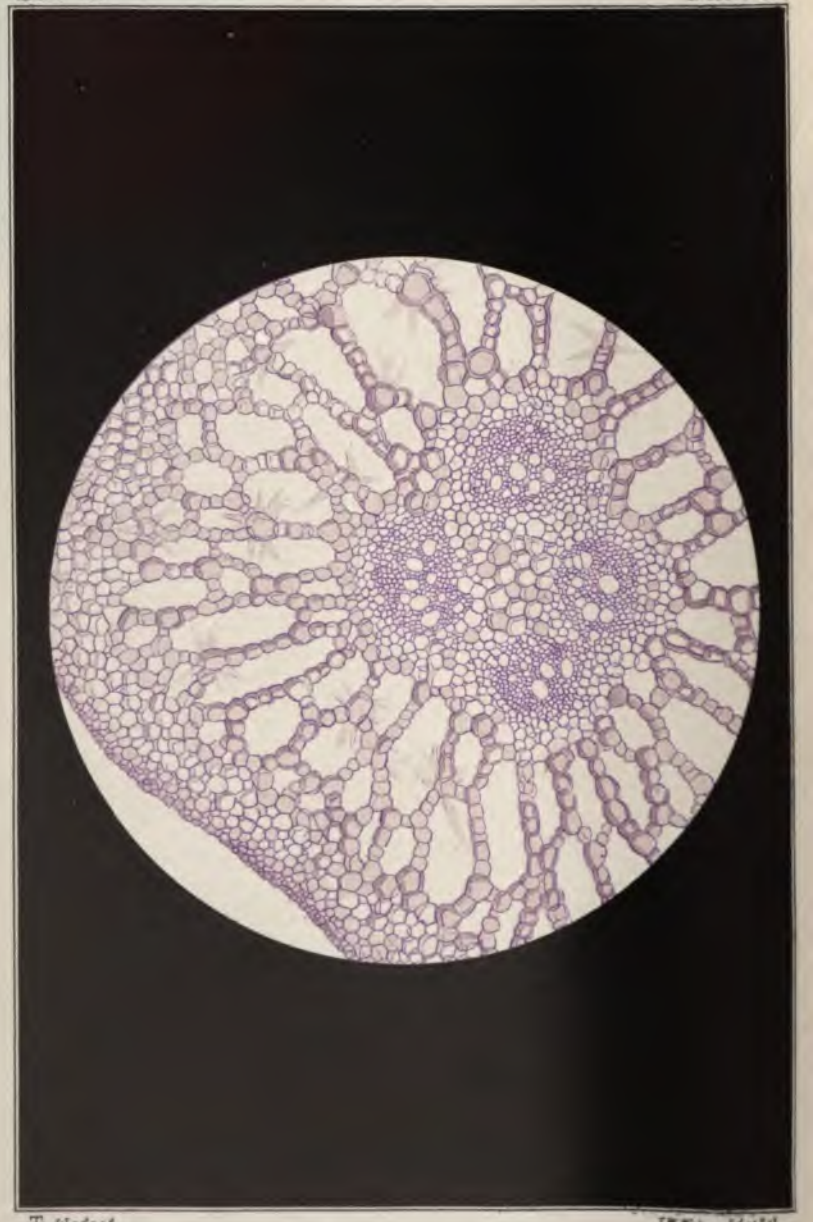
„ 2. Longitudinal section of the margin of a principal vascular bundle of common Bracken Fern *(Pteris Aquilina)* at the place where the thickening layers begin to appear in the spiral vessels. *After* HOFMEISTER.

„ 3. Transverse section of wood cells of young stem of Sunflower, showing "middle lamella" and thickened portions of wall. *After* SACH.

„ 4. Longitudinal section of stem of *Italian Reed*, the cortex lies towards the right of the bundle, and the pith towards the left. *After* CARPENTER.

„ 5. Detached woody fibre of *Pinus* showing the bordered pits.

„ 6. Longitudinal section (somewhat diagramatical) of wood of *Pinus* showing the nature of the "pits."



del. et lith.

T. S. PETIOLE OF LIMNANTHEMUM.

x 75

Watson & Son Lith. 53 St Charles St Boston

FUNDAMENTAL TISSUE.

"Fundamental" is a term which has been applied to all permanent tissue, not referable to either of the two systems we have just been studying—epidermal or vascicular. In its simplest form—such as we find, for instance, in the accompanying preparation—it consists merely of thin-walled parenchymatous cells, functionally useful in the conveyance of assimilated material from one part of the plant to another, and for the storage of starch and such like reserve material in organs that are destined to enjoy a biennial or perennial existence. As this system forms a ground tissue through which the isolated fibro-vascular bundles ramify, it is, in the stems of Dicotyledonous plants, generally separable into three regions—the pith or medulla, cortex and medullary rays. So far as their comparative extent of growth is concerned, these regions vary very much in different plants. Compare, for example, the smallness of the pith and the wide region of cortex in the section about to be examined, with the thick pith and narrow band of cortex as displayed in a transverse section of the sunflower—and the exceedingly narrow medullary rays in Scotch fir, with the broad ones so clearly seen in the section of the stem of the cucumber.

In roots—as typically displayed in dendrobium—owing to the axial position of the fibro-vascular bundles, the pith is never large, while even that, small as it is, is subject to complete obliteration if the roots eventually increase very much in thickness.

In most leaves the fundamental tissue is largely developed, while the cells contain an abundance of chlorophyll, and are therefore functionally different from those to whose contents light cannot readily penetrate.

In aquatic plants, the cells of the fundamental tissue are not so closely arranged as in those of the various land plants, sections of the stems of which we have already examined. In all the aquatics, large or small intercellular spaces, bounded by plates of cells, are invariably left in the ground tissue, thereby providing a convenient receptacle for the storage of air required either for purposes of respiration or to give buoyancy to the various organs of the sub-aqueous plant.

The subject of our present study is a transverse section of the petiole of a species of *Limnanthemum*, logwood stained, and drawn under a magnification of 75 diameters. It is selected to illustrate unmodified

fundamental tissue, intercellular spaces and idioblasts. Limnanthemum is an aquatic genus of exotic plants belonging to the same family as our own bogbean and gentians. The petioles are long, slender, alternate and submerged, and carry round heart-shaped leaves, and umbellate heads of small, regular flowers.

The following points of structure may be observed in the section :—

(*a*) The *epidermis* of small, thin-walled cells scarcely differing (except in size) from the underlying cortical cells.

(*b*) The *vascicular system* made up of four closely arranged axial bundles, each presenting the cut ends of several wide vessels (*spiral vessels as may be seen in longitudinal section*) surrounded by cells, the walls of which are thin and unlignified.

(*c*) The *fundamental tissue* consisting of (1) a small region of *pith ;* (2) short but comparatively broad *medullary rays;* (3) the *bundle or plerome sheath*[1] a distinct hoop or line of cells,—thin-walled and containing grains of starch—running round the axial vascicular system, curving over the convex outer end of each fibro-vascular bundle and dipping into the medullary rays between the bundles ; (4) the *cortex* composed of an outer deep region, and an inner narrow region of closely arranged sub-globular, thin-walled cells, and a wide breadth lying between these, where the cells are disposed in radially long and tangentially short reticulated rows, kept apart by the existence of comparatively large intercellular spaces.

(*d*) The *idioblasts* (or internal hairs) isolated, stellate cells scattered throughout the intercellular region of the cortex.

It will be noted that the petiole of Limnanthemum being a submerged organ, it is in consequence not subject to the trying and ever-varying vicissitudes of a sub-aërial life, and that, as might be expected, a functional epidermis is found in this preparation to be all-but entirely absent.

It will be also noted that the position of the fibro-vascular system in the petiole of this plant is similar to that observed in roots (compare with Dendrobium), that is, it is axial ; and that still further, as in roots, there is a distinct plerome sheath enclosing the vascicular system. In common bogbean (Menyanthus) above referred to *each* bundle is enclosed in a sheath, a point of structure in which it departs from typical Dicotyledons, but which is very characteristic of Ferns, and perhaps the majority of all other vascular cryptogams.

[1]This point of structure is, unfortunately, not clearly shown in the accompanying drawing.

In our type, as in water plants generally, we find a very poorly developed prosenchymatous system ; but remembering that functionally the woody fibres are concerned, first, in giving mechanical support to the plant ; and second, in conveying water from root to leaf, the non-advantage of lignification in these tissue elements in the water-covered organs of aquatic plants is, of course, easy to fully understand.

The fundamental tissue, or rather, perhaps, certain regions of it, in the majority of land plants are subject to many adaptive modifications, the exact nature of which is, or has been, determined by the external conditions to which the plant or its progenitors have been subjected. For example, in the long succulent, rapidly growing stems of cucumber, a clearly differentiated cylinder of thick walled cells (the *hypoderma*) forms in the cortex (as we have previously seen) a region of strength in the otherwise weak and generally soft-celled leaf-bearing axis. Similar hypodermal regions are very common in stems, and we may mention the well-known meadow-sweet as a good example wherein this modification of the fundamental tissue system is tolerably well represented.

In some cases the modification reaches extreme limits, as is exemplified in the formation of such hard tissue as the fruit-stones of plums, cherries, and the like. Such intensely strengthened tissue is known as *sclerenchyma.*

A modification of the fundamental tissue of a totally different nature is characteristically displayed in the transverse section of the stem of cucumber (slide No. 9). If the isolated groups of cells lying within the circumferential ridges of this preparation, referred to in paragraph (*c*) in the table of instructions given on page 36, are carefully examined under a high magnifying power, it will be discovered that the description of "thin-walled," as therein applied to the cells, is, at best, only partially accurate, as, by a proper adjustment of the light, it will be observed that the angles formed by the meeting of the lateral or longitudinal walls are filled with a clear-looking, finely striated thickening material, the presence of which reduces the cavity of each cell to an almost circular form in sectional outline. This special kind of tissue is known as *collenchyma ;* it possesses the power of swelling to a greater or lesser extent by the absorption of water ; while functionally it is concerned in giving strength and a considerable degree of elasticity to the organ within which it is generated. The cells are, generally speaking, moderately long ; they are filled with sap, and contain either only a few grains of chlorophyll or none at all. The cell-walls may be coloured a bright blue by submitting sections to the action of chlor-iodide of zinc.

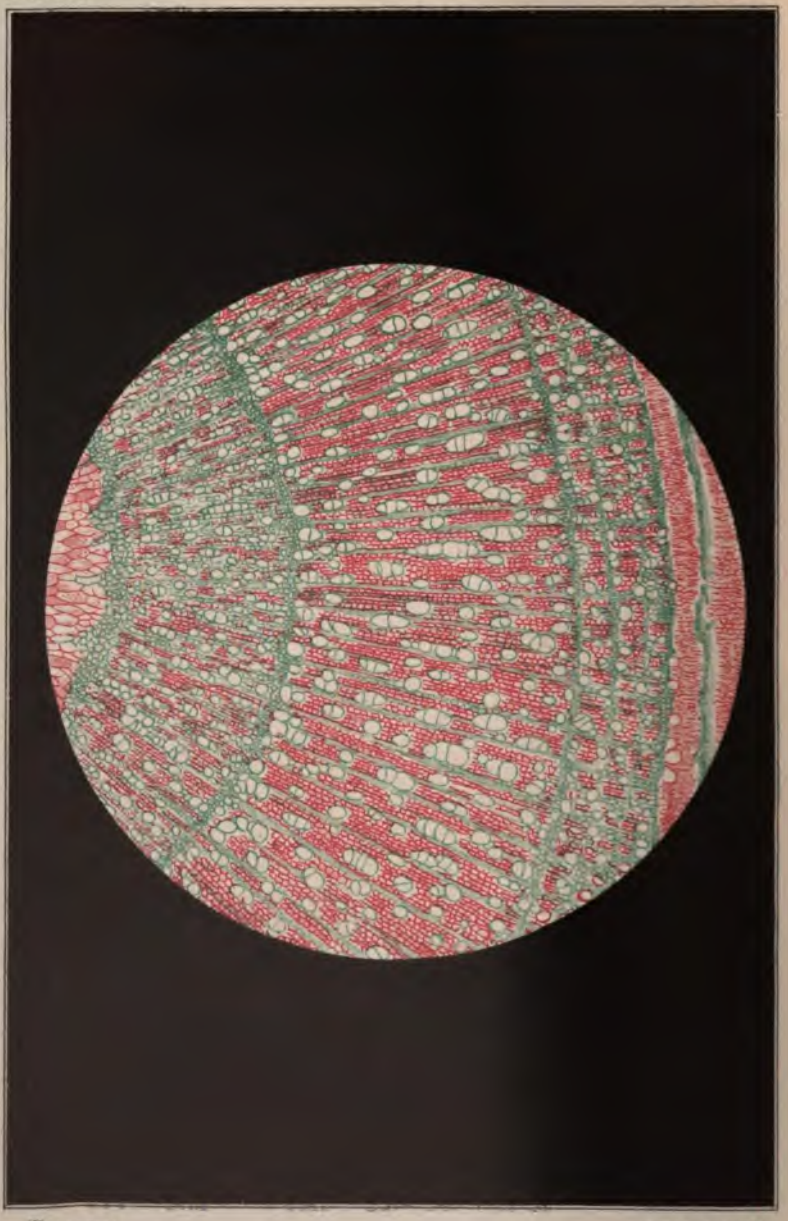
Although the occurrence of collenchyma is comparatively rare in Monocotyledons, yet it seems to be generally present in all the climbing species, such as *Smilax*, or that commonly cultivated Chilian plant with crimson flowers and edible berries—*Lapigeria rosea.*

The function of the intercellular spaces in Limnanthemum has been already referred to, but, for the sake of comparison, we may here again direct attention to the intercellular spaces in the stem of the Scotch fir. As has been already stated on page 11, the intercellular spaces in this plant take the form of canals, and act as receptacles for the resin secreted by the cells that form the walls of these canals.

In the Umbelliferæ we find intercellular spaces, containing a mixture of resin *and* gum, while in other cases—the water plantain *(Alisma)*, for example—the canals are receptacles for a kind of latex.

In the fundamental tissue of the leaf, intercellular spaces occur that are placed in direct communication with the atmospheric air through the medium of those important epidermal intercellular spaces—the stomata.

Finally, with respect to the idioblasts (SACH'S term for "individual cells in a tissue otherwise homogeneous" that "become developed in a manner strikingly different from their neighbours"), we have seen that those in Limnanthemum bear a striking resemblance to hairs—hence their special name of *trichoblasts*. As examples of other forms of idioblasts we may refer to the *lithocysts*—comparatively large cells, containing clusters of crystals (*cystoliths*)—typically displayed in the hypodermal cells of the leaf of the Indian-rubber tree (*Ficus Elastica*) or to the laticiferous idioblasts as exist in the tissues of Spurges (*Euphorbia*) and other plants.



E.T. del. et sculp.

J. W. Watson del. et lith.

TRANSVERSE SECTION STEM OF MAPLE.

Shewing annual rings.

X 50

Watson & Son Lith. 83, St. Charles St. Berm.

SECONDARY TISSUE.

In all perennial structures, provided with open fibro-vascular bundles, fresh growths annually arise through the persistent activity of the meristematic cells of the cambium. These secondary layers of wood and bast may be observed in almost any woody-stemmed Dicotyledon, but a three-year-old branch of the common Maple *(Acer Campestre)*, in transverse section and double stained, has been selected as a typical example of such secondary increase in thickness.

Early in the history of the branch, and while it was yet in the bud stage, the first-formed or primary bundles arose directly out of the meristem of the apical region, after the manner previously described herein. These bundles were of course open, and the layers of cambium lying between the regions of wood and bast *(fascicular cambium)* were connected by similar plates in fundamental tissue *(interfascicular cambium)* so as to form a complete cylinder of active meristematic cells. As the bud lengthened into a branch its axis increased in thickness through the additions made to it by new cell formation in the cambium. Fresh layers of wood and bast were formed by the interfascicular as well as by the fascicular cambium, and as a consequence the primary wood appears as so many processes outstanding in the pith, while the primary bast bundles get more and more widely separated from one another, as they are outwardly pushed by the thickening stem. One histological difference between the primary and secondary wood is that in the former the vessels are spirally marked, while in the latter they are deeply pitted. Owing to this difference the cylinder formed by the primary system is descriptively known as the *medullary sheath.*

During the later weeks of the growing season, and when vital energy is on the wane, there is a marked difference in the size and diameter of the xylem elements, therefore, when, in the next returning Spring, the cambium again becomes active, the comparatively large and normal tissue elements stand out conspicuously beside the dwarfed growths of last autumn, so that a clear and well-defined boundary marks the line where one year's growth ends, and another begins.

Furthermore, in structures that are destined to withstand subærial exposure for years, the ordinary epidermis is early replaced by a more efficacious protective layer of squarish cells *(cork)*, containing (when mature) nothing but air, and whose walls have suffered a characteristic modification. The material *(suberin)* of the cell-walls, while being very elastic and extensible, is most resisting, standing, as it does, without solution, the action of concentrated sulphuric acid. Water also is unable to pass through it. Physically and chemically, therefore, cork is eminently fitted to act in the capacity of an external protective tissue.

Before examining the section it will be well to procure a twig of Maple and make a few careful observations with the naked eye. Selecting there-

fore a bit of a three-year-old twig, it will be seen that, externally, it is covered with a dry, dark-brownish flaky "bark" with pimple-like excrescences *(lenticels)*, scattered pretty freely over its entire surface. Owing to pressure from below, the "bark" is split longitudinally into narrow anastomosing bands, exposing an ashen-hued investment beneath. The lenticels are formed of a collection of loosely arranged cork cells that take the place and perform, in many young stem structures, the functions of stomata. By scraping the outer coat away the underlying ashen-grey layer may be fully exposed. It is a cylinder of cork, papery in texture and known as the *periderm*. If this layer is in turn carefully scraped away, a green layer of a parenchymatous nature may be easily displayed. Using the knife again, but this time very carefully, a number of very fine white threads will be discovered lying in softer tissue immediately beneath the layer of green cells. The threads are primary bast fibres, and the succulent tissue the soft bast. By continuing the scraping, broad strands of secondary bast will be exposed that form altogether an almost continuous cylinder, around the stem. This cylinder is very easily detached from the internal shaft of wood, and when so removed the outer surface of the wood and the inner surface of the bast present a smooth moist appearance. The moisture is due to the spilt contents of the smashed cells of the tender cambium that lay between. By cutting the wood away on one side an internal mass of pith may, lastly, be laid bare.

Turning now to the section issued herewith, the following general points of structure can be made out with an ordinary lens:—

1.—A central disc of pith (crimson stained), made up of comparatively large roundish cells.

2.—A broad ring of wood (green stained) clearly marked out, by two dark, thin-lined circles, into three regions (representing three years of growth). The dark ground tissue represents the individually narrow and closely-arranged woody fibres while the clear specks are, of course, the cut ends of wide tubes, or wood vessels. A dark, wavy tissue-band lies to the inner side immediately surrounding the pith.

3.—An external circular band of "cortex" (stained red), with three or four narrow, isolated, interrupted hoops (of a greenish-yellow tint) occurring within its substance. The outer or circumferential layer (brownish hued) is burst, and shows a tendency to "peel."

4.—Numerous slender lines—medullary rays—starting from the pith and passing through the wood and cortex to the circumferential layer.

By using an 1-inch obj. the following particulars can be made out with respect to the various systems of tissue:—

1.—The *pith* of roundish or polygonal cells becoming larger and laterally compressed towards the circumference.

2.—The *wood* or *xylem system* made up of densely packed, narrow cells (woody fibres) interspersed with isolated or associated (in twos, threes, or even fours) wide tubes or wood vessels. The woody processes(primary xylem) that are pushed into the pith and the boundary lines between the annual growths are formed, as will be seen, of much narrower tissue elements than those found in the rest of the same system.

3.—The *cambium* represented by an often contorted (becoming so during manipulation) band of cellular tissue that has taken up very strongly the carmine stain ; the outline of the individual cells may be perhaps just made out in parts where contraction has not taken place.

4.—The *cortex* (so named here for convenience) made up of (*a*) hard and (*b*) soft bast, (*c*) green layer, (*d*) younger or inner, and (*e*) older or outer layer of cork.

(*a.*)—The hard bast, comprising an outer series of green-stained patches (the primary hard bast) concentrically arranged, and three inner greenish or yellowish-stained more or less continuous circles.

(*b.*)—The soft bast of thin-walled, carmine-stained elements, in which the interrupted hoops of hard bast are imbedded.

(*c.*)—Green layer of rather larger and tangentially compressed cells, the chlorophyll of which has been dissolved out, and the walls and contents carmine stained.

(*d.*)—Younger suberous layer composed of rectangular cells, the walls of which are slightly stained with green.

(*e.*)—Outer suberous layer made up of irregularly compressed or contorted cells passing into an amorphous brownish superficial layer of varying thickness.

5.—The *medullary* rays, some *(the primary)*, commencing at the circumference of the pith, others *(the secondary)* commencing in the wood of the second and third year.

Under a quarter-inch obj. the following details may be observed :—

1.—In the *pith*, clusters of starch grains may be discovered in some of the cells, but in the cells of the two or three outermost layers of all (which are roundish in outline, and have taken up the green stain, appearing therefore under a low power as belonging to the xylem system) starch grains completely fill up the cell-cavities.

2.—In the *xylem system* may be noticed the difference in diameter and outline between the woody fibres in the primary and secondary growths, in the regions marking the boundary between the annual layers and in those touching or bordering upon the vessels.

3.—In the *cambium* region the thin-walled rectangular meristem cells may be seen regularly disposed in radial rows, the protoplasmic contents being stained a deep crimson.

4.—The *hard bast* elements are displayed as closely set angular fibres, with walls so completely thickened as to leave an extremely narrow internal cavity. Faint striations may be distinguished in the substance of the walls. Compare with fibres of xylem.

5.—In the *soft bast* the sieve plates of some of the phloëm vessels may be discovered by careful search and focussing.

6.—The *medullary rays* in the wood are filled with minute grains of starch. In the bast region they appear to be empty. The individual cells are generally larger (at least towards their terminations) in the bast than in the wood.

7.—The cells of the *green layer* are large and tangentially elongated (oval in sectional outline), and their contents are granular and crimson stained.

8.—The *inner suberous layer* is made up of square or sub-rectangular cells, disposed in radial rows. It is clear-looking as the cells are empty.

9.—The *outer suberous layer* is made up of distorted and disorganised cells, portions of which are lying on the circumference as brown, structureless masses.

It may be noted, as points that may be made out in longitudinal sections of the fresh stem, that the cells of the pith are dotted, and that the vessels of the primary wood are provided with spiral thickenings, while those of the secondary growths are closely pitted. The woody fibres run off to a point by an one-sided slope, after the manner of the cutting edge of a chisel. The bast fibres are very slender and very long. The cells of the green layer are a little longer than their greater breadth, the walls are thick, clear-looking and homogenous. The inner cork cells are almost square, their walls are thin and their contents are merely air. Chlorophyll is not only found in the green layer, but it pervades the cells of the medullary rays, and is even (in one and two year old twigs) found in the outer starch-bearing cells of the pith.

This preparation will, it is hoped, sufficiently demonstrate the normal mode of increase in thickness in dicotyledonous stems, by the formation of secondary vascicular tissue through the activity of cambium. Secondary increase in roots is effected by a similar process of new cell development from a layer of cambium cells. There is an initial difference, however, as to the origin of the cambium. It will be remembered that in roots the bast and xylem bundles are arranged radially, and not collaterally as in stems ; now the cambium from which the secondary vascicular tissue is generated arises in the parenchymatous tissue behind the phloëm masses only, and very soon, owing to its activity, new or secondary wood is formed on its inner or medullary side, and secondary bast on its outer, or against the inner side of the primary phloëm. A cambium layer appears in front of the primary xylem bundles, but this will merely produce parenchymatous tissue to keep pace with the thickening of the stem by woody increase. From this date increase in thickness goes on just as in stems, and the secondary wood may or may not subsequently coalesce with the primary.

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APPENDIX.

SECTION II.

BOTANICAL HISTOLOGY.

PAGE 29.—Lines 7 from top, 9 from foot, and 5 from foot; for "stomatae" read "stomata."

The same error occurs several times on pages 30 and 31.

PAGE 38.—l. 16 from top; for "it cover the whole surface" read "it covers the whole surface."

PAGE 50.—l. 22 from foot; for "homogenous" read "homogeneous."

proceed to Loch Thamana-bhaidh, as the nearest harbour *south* of the Islands, and here lay in a good stock of patience. If it has been blowing gently for say four days from W.S.W., make your first venture. Sail round the islands, examining the spots where your captain says landing can be made. Probably you find a surf rushing fifty feet up the cliffs. Should the wind run round to the north, seek harbour in Loch Carloway, Loch Roag; in order to hold a vantage start. Here lay in a second stock of patience, but hold self and crew ready to turn out, even at the moment they feel most ready for turning in.

Should it fall "flat calm" when you are within six miles of the islands, during your next venture, and you feel that you must draw heavily upon your stock of patience, do so by all means; but spice abundantly with determination, and keep hanging on day and night by the islands, for your chance is now or never for a month. If on jumping ashore after your companions you come down *crunch* on one knee, you be dragged by those companions half fainting beyond the sweep of the second surge (the first has gone over you to the neck), you lie for a fortnight on the deck of the yacht with your knee strapped in bandages, and of the colour of a copper Indian from tincture of iodine, and if you carry a notch in your kneepan to your grave, still be thankful—*you have got your specimen.* You may have escaped worse things than a notched kneepan; especially if it may have chanced that your pilot was the same as he who had charge of the Lively when she lately tried conclusions with the Chicken.

How to Prepare a Rock Section for the Microscope.

It is not necessary to procure expensive slitting and grinding machines for this purpose. In the production of a large number of sections elaborate instruments save time and labour, but for all ordinary work, such as the preparation of a dozen sections or so, a few simple tools will be found quite sufficient. Let us see how we can prepare a good section without the aid of a lapidary's bench.

The following articles should be procured:—1st. A good geological trimming hammer and chisel.[1] 2nd. Two thick zinc or lap-metal plates, each about 12 × 15 inches in surface, also a slab of plate glass of the same size. 3rd. A Water-of-Ayr stone, or a slab of marble. 4th. Emery powder of three kinds—(i.) Oakey's No. 50 or No. 60 hole; (ii.) Oakey's No. 90 hole; (iii.) Oakey's finest flour emery. 5th. A few bits of stout window glass cut into pieces of about an inch square each. 6th. Some hardened Canada balsam, made by heating ordinary liquid Canada balsam in a sand or water bath, until on cooling the balsam become as hard as ice; it may be put up in the form of rods of sealing wax. 7th. Canada

[1] These may be obtained from Mr. Russell, 48, Essex Street, Strand, London, W.C.

POPULAR MICROSCOPICAL STUDIES.

HEBRIDIAN GNEISS.

From the Flannan Islands, × 25 diameters.

f. Felspar; *q.* Quartz; *h.* Hornblende.

On the Hebridian Gneiss of the Flannan Islands, by M. FORSTER HEDDLE, M.D., F.R.S.E., ETC., Professor of Chemistry in the University of St. Andrew's, Past-President of the Mineralogical Society of Great Britain and Ireland.

It is always well to begin at the beginning. Sections of several rocks will, from time to time, be laid before our contributors; for rocks, equally with the petals of flowers, the wings of insects, and the feathers of birds, are things of beauty;—perhaps more than these do they disclose the genius of constructive power, the adaptation of means to an end. We begin at the beginning, that is, with the oldest rock,—the first brick of the edifice,—the deepest film of the crust of the earth. We know down through or into that crust for about twenty-eight miles. Not that we have ever bored a hole nearly so deep as that, or could have gone to the expense of so doing to supply an illustration; but that convulsions and elevations and denudations have tossed up, or brought within our reach, portions which originally lay even so deep down. But, to be perfectly precise, it has to be said that the rock which forms the Hebrides, conferring upon these barren islands their sinuous coast lines, and their rugged crests, is not altogether and absolutely the deepest known of any part of the earth's surface, though it belongs or pertains to that which is. That oldest rock is found in the Gulf of St. Lawrence in Canada, and this rock of the Hebrides is a little higher in the scale—a kind of *upper crust* to it. It is so old, however, so deep down, that GEIKIE happily has called it the "Floor of Europe;" and more happily, and with prophetic prevision, our forefathers commonly, and somewhat jauntily, but with dignity profound, were wont to speak of "Old Scotland;" and is it not now generally admitted, that, if not an uneducated man, he is an

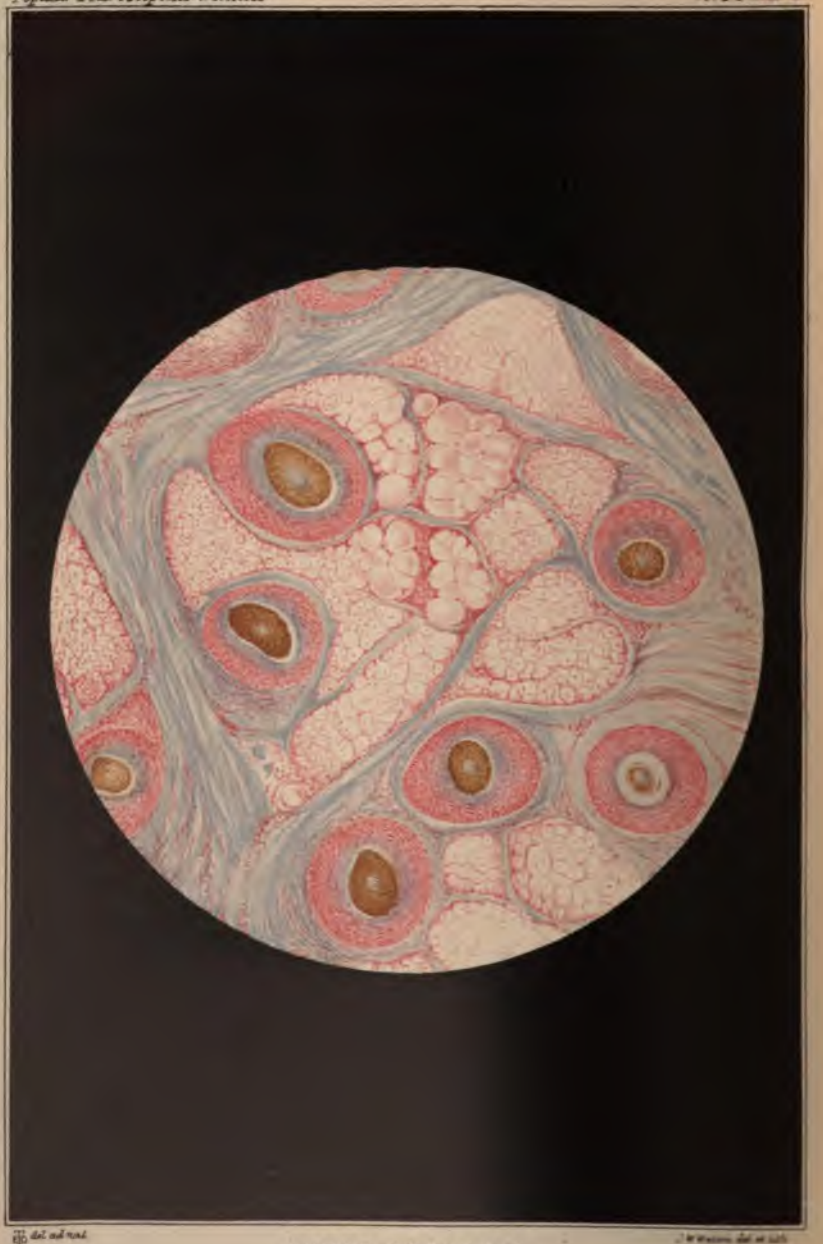
thoroughly washed in water, and still further reduced on the next plate with finer emery (No. 90 hole) until it is tolerably transparent and of uniform thickness throughout.

7TH PROCESS.—Rewash well in water, and progress carefully with the grinding on the glass plate with fine flour emery. During this process the section ought to be constantly examined under the microscope until the desired degree of thinness has been attained, when it may be finished off with care on the Water-of-Ayr stone or on the smooth marble slab. In the case of the Flannan Islands Gneiss, the muddy-green material, or hornblende, ought to appear clearly greenish, and when placed under a singly rotating Nicol's prism ought to be seen to change in colour to a brownish hue; if the crystals appear black instead of green, then the section is not thin enough.

It is sometimes useful to have both thin and thick sections, but, as a rule, the sections ought to be transparent enough to enable the observer to distinguish ordinary printed letterpress through them. The sections may combine thin and thick parts; it is always easiest to prepare them thus; to have the edges thin and the central portion thicker, and these are more useful than uniformly cut sections, where only one specimen is available. To attain uniformity of thickness, when the centre happens to be perversely thick, a slightly convex grinding surface, such as a turned disc of wood or metal, may be used with the finest flour emery, or with jeweller's rouge, or rotten stone.

8TH PROCESS.—Pour a little benzol into a saucer, or better still, into a white porcelain saucer-shaped artist's palette.[1] Gently heat the square piece of glass with the adherent section over a Bunsen or spirit flame; as soon as the hard balsam has been melted, the section may be removed with the edge of a penknife into the benzol, and allowed to soak therefor about five minutes or more. A soft camel's hair brush should be used as a lifter; with a little care the section, however thin, may be transferred on to a glass slip into a drop of balsam, and covered in the usual way with a thin covering glass.

[1] These palettes, which are far more useful than watchglasses, may always be procured from Messrs. Rowney, of 52, Rathbone Place, London, W.



HUMAN SCALP H.S.
X 130.

Weston & Son Lith. 9 Charles St Bath.

POPULAR MICROSCOPICAL STUDIES.

THE SCALP.

HORIZONTAL SECTION OF HUMAN SCALP.

DOUBLE STAINED.

× 130 Diameters.

Etymology.—Scalp, or Skalp, *n.* (*Shelp*, *Schulp*, in Dutch), meaning literally the skull; cranium; brain-pan; also, that part of the integument of the head usually covered with hair; hence, the skin of the head, or a part of it, with the hair belonging to it, torn off by North American Indian warriors as a token of victory.

INTRODUCTORY REMARKS.

If we look upon the skin of the head merely as skin, with or without hair covering it, this is exactly as we ought to do: it is really only integument, such as is found all over, or nearly all over, the body, under a modified form. It has all the properties of skin, and its hair has all the properties of hair, but these are in many ways peculiar. For example, no part of the skin of the body, except that of the head, can be moved upon the parts beneath to any perceptible degree by any mechanism specially attached to it. If the reader has noticed a fly alight on the flanks of a horse, he will have observed the skin of the flank quiver, and the fly dislodged. The skin in this region has the power of movement, not residing within itself, but a sheet-like, delicate muscle, called the *panniculus carnosus*, has the power of vibrating the skin to and fro. This is so in many quadrupeds. In human beings, there is a muscle beneath the skin of the head, which can move the skin backwards and forwards. This is called the *occipito-frontalis*, because it is attached to the occiput at the back of the head, and to the frontal bone at the front of the head. The scalp sits quite loosely upon the bones of the skull,

with this muscle between the two. In most people, this muscle is never exercised, so that it remains thin, and weak, and quite incapable of drawing the skin of the head backwards and forwards, except to a very limited extent. The human ear lobes have also two or three little delicate muscles, which man, having his hands always at the ready, never exercises. Now, with perseverance in their regular exercise, these little muscles of the scalp and the ears can do their work very efficiently, indeed. The writer has seen an old Edinburgh professor, who used to insist that most muscles were under the control of the *will*, if one liked to exercise that will, who could and did demonstrate to his class every winter this fact in a remarkable manner. He would say:—"Gentlemen, Lord Dundreary asks his brother Sam if he (Sam) can wa-wa-wag his left ear." The class would be in a roar to see the old professor's ears going like flappers, and his scalp brought almost over his eyes, then drawn quite as far back as it had been drawn forward, to the unbounded delight of his audience. Another peculiarity of the scalp is its great vitality. If the skin of any other part of the body be severed, the chances are ten to one that it withers and dies. With the scalp, however, this is not so. In accidents people sometimes get scalped as effectually as a Red-skin would do it for them, such as by the wheel of a cart or carriage running over the head. Now, an enterprising surgeon, would quickly wash the scalp free of dirt and place it upon the skull, and with care and the aid of suitable dressings he would expect it to adhere, and in a perhaps low percentage of cases, his expectations would certainly be realized. Other peculiarities, such as baldness, loss of its hair during or after certain illnesses, &c., will be referred to subsequently.

The Hair.

The hair of the scalp has also its peculiarities, but we will not stop to treat of these here, but at once proceed to consider the common characters of hair, whether on the scalp or elsewhere. A hair, then, consists of :—

1. A Root,
2. A Shaft; and
3. A Point.

The root of the hair is lighter in colour and softer than the stem. It swells out into a bulbous enlargement or knob, and is received into a recess of the skin named the *hair-follicle*. We will pass over this at present, as it will be better demonstrated in a subsequent issue of a vertical section.

The *stem*, in its transverse section, is well seen in the accompanying lithograph. The stem is usually cylindrical, especially as it emerges from its pit, or follicle, but in natural hair, growing to its full length, and not interfered with by the razor or scissors, the stem becomes gradually smaller towards the point. The length and thickness vary greatly in individuals: in the different regions of the body; also in the various

races of mankind. In the straight-haired races, the individual hairs are coarser and thicker, and the section more circular than in the woolly-haired races, in which the section is smaller, and oval, the hairs being sometimes markedly flattened. The section is largest in the North American Indians, Chinese, and especially in the Japanese; light-coloured hair being usually finer than black. The stem, again, is surrounded by scales, which lie upon the surface exactly like the slates on the top of a house; that is to say, one scale overlaps another. Within this scaly covering is a fibrous substance, which in all cases constitutes the chief part of, and, often, the whole stem, but in many hairs the axis or centre is hollow, and occupied by a kind of pith. This is so in our present section. The outer coat of scales is stained with carmine: the middle fibrous coat is a very pale blue, whilst the centre cavity can be well seen to contain a brownish, granular-looking mass—the pith, or medulla. The latter, in some cases, fills the cavity, but in others it has shrunk, and left the sides. The fibrous substance is translucent, and may be broken into long fibres, which, when separate, are found to be flattened. These fibres, again, may be further found to be made up of flattened fusiform cells. Very slender elongated nuclei may be seen, also specks containing air, caused by minute cavities. These air spaces are abundant in white hairs. The medulla or pith does not exist in all hairs. It is absent in the fine hairs covering the body. When present, it occupies the centre of the shaft, and ceases towards the point. It is seen to be darker than the fibrous part when viewed by transmitted light, but by reflected light it is white, its colour being due to particles of air. It is composed of rows of soft cells, containing fine granules and air.

Viewed with a low power, it will be further observed that the hairs and their follicles are in groups of three and four, more rarely two, and very occasionally they are single. Each hair, with its follicle, has accompaniments, of which the most conspicuous in our present section is the sebaceous gland. Transverse sections of these glands are very beautiful, here every cell of the gland and every nucleus within its cell being mapped out by the delicate staining reagents. As we see these sebaceous glands now, they appear round or pear shaped, according to the way the gland has been cut. These glands are like pears in shape, the stem of the pear being uppermost, and the body hanging down, swinging, so to speak, by the side of the follicle. Therefore, if we look at one cut through the "stem," we are really looking into the mouth of the gland, and we find it round and of small diameter. There are very few in the present instance: the majority of the glands are cut across their middle, especially the upper part of the middle—the depth of the section just allowing the bottom of the gland to be present, and this is seen almost without altering the focus of our low power. Besides these sebaceous glands we see minute bundles or masses of fibres, quite irregular both in shape and size, stained a very pale blue.[1] Small *staff* shaped nuclei stained red reveal the nature of these little bodies; they are parts of the minute muscles of the hair follicles or muscles of

[1]The artist has chosen a part of the section where these are quite absent

the hairs. These will be seen at length in the subsequent vertical section as slender bundles of plain muscular tissue connected with the hair follicles, but generally arising by a number of very small bundles from the under part of the corium. In other words, every hair has its little muscle to "make it stand on end," and every little muscle arises or takes its origin, or fixed point, in the under surface of the corium; these minute origin bundles then unite: pass obliquely downwards and as one bundle, take insertion into the outside of the pit or follicle holding the hair *below* the sebaceous glands. Of course, in our present section, that part of the shaft (so to speak) of the muscle is cut across which is on the same level or plane as the sebaceous glands; therefore the fibres and staff shaped nuclei of the muscle are in focus together with the sebaceous gland cells.

Again, every group of hair follicles, etc., is seen to be surrounded by open wavy fibres which are loosely woven around each group and stained a very pale blue. If we observe carefully we shall see parts of these fibres interlacing within each group, dipping into each group, and twining around each component of the group whether hair follicle, or gland. Most fibres are seen to surround the hair follicles; we trace them with greater difficulty as they surround the glands. These fibres go to form the areolar tissue as it is technically called. This tissue holds the same relation to the other parts we see here that the racks of a wine bin hold towards the bottles. It acts as a support to the hair follicles, sebaceous glands, blood vessels, nerves, etc. The spaces in this tissue communicate freely, and allow hair washes and other medical applications to reach the roots of the hair, the blood vessels and nerves of the scalp. The spaces of this tissue do not exist in the natural condition of the body, but the whole tissue forms one unbroken membrane. The chief use of this cellular tissue is to bind parts together as in the present case, whilst by the laxity of its fibres and the permeability of its spaces it allows parts to move freely on each other, and affords a valuable space for the escape of "waste humours" and inflammation products. This cellular tissue consists of two kinds of fibres, white and yellow. The white fibres are easily distinguished, as they run in wavy bundles, which interlace with one another. The yellow fibres are larger than the white, and branch and anastomose freely with one another. The great elasticity of the skin everywhere is due to this cellular tissue. If, for example, a cut be made in the fresh skin, as it lies on the body, the edges of the cut immediately gape, and the surgeon has to draw them together with a suture or to unite them by means of sticking plaster. This cellular tissue is very interesting to lay readers who have had experience of medicines injected under the skin instead of being taken by the mouth. It is into the meshes of this tissue that the point of the syringe penetrates. In a few seconds the medicine traverses every part of the body, because this areolar tissue is universally distributed: the fibres of muscles: the various organs, nearly every part is connected by this tissue.

del. sol. nat. J. W. Watson. del. et lith.

HUMAN SCALP

Vertical Section, double stained.

X 25.

Watson & Son, Lith. 313 Charles St. Bmo.

THE SCALP.

Vertical Section of Human Scalp.

In the Stained.

× 25 Diameters.

In continuation of our remarks on the scalp, which was seen in horizontal section, we now go over what was said and draw attention to the various details observable in this vertical section.

We saw that the hairs of the scalp were in groups mostly of three, more or less. We also saw that connective tissue was interwoven among the groups, and dipped into each group and surrounded its constituents; and we further were able to distinguish the various parts of the stem: its outer epithelial covering, its dermic portion, and its hollow interior more or less filled with pith.

It was observed that the hairs were each composed of a root, a shaft, and a point. In neither preparation can these all be seen, for obvious reasons: the point has, of necessity, been cut away in the horizontal section, whilst in this vertical section no hair is short enough to be included. The root of the hairs is well seen, as a fair percentage have been just in the plane of the section, whilst others have been above the plane of section, as the piece has lain in the well of the microtome, and have, therefore, been wholly or in part cut away.

Now, we see the hair follicle, or pit in which the shaft and root of the hair are placed, in perfection. The hair shaft and root just fit the follicle, except around the root, where the follicle is not quite in contact with it. These follicles are really the skin of the scalp turned inwards, so to speak, as we can see even with a low power: the part nearest the hair shaft and root is epidermic lining *continuous* with the cuticle, whilst the remainder is dermic and continuous with the corium. Just at the surface the follicle is wide and not in contact with the hair. This part is called the mouth of the follicle. The part where the lowest end of the mouth joins the part in close contact with the hair shaft is called the neck of the follicle. If we divide the hair follicle into four equal parts or quarters, we see that the sebaceous gland of the follicle occupies the length of the two middle quarters—that is to say, the sebaceous gland is half the length of its corresponding follicle. We have now the opportunity of seeing the shape of the sebaceous gland: it is pear-shaped, as

was before pointed out, the stem of the pear being uppermost, and now seen to be the gland duct opening into the follicle about one fourth of the distance from the skin surface. Allowance must, of course, be made for the planes of the section we are viewing being uneven—thus, whilst one plane may by chance be parallel with an entire gland, and shew the whole gland, another section may have a different plane, and we may only have the stem of the cells composing the comparison), or the part of the body of the pear remaining. In the latter case, we should see the cells composing the gland at a distance from the corresponding follicle, and having no apparent connection with it whatever. In the section the writer has under a low power (1 inch) of his microscope there are about twenty hair shafts and follicles, or parts of these. It is difficult to count them, as some appear in two parts, upper and lower, two bits of shaft, or one bit of shaft and a bit of root, and so forth. It must, therefore, be remembered that for descriptive purposes an author in writing, and an artist depicting by a drawing all the parts of a hair and its surroundings, must either be uncommonly lucky in possessing a section having a plane parallel in its entirety, with all the parts he wishes to depict, or he must *make up* a diagram, construct it from the bits and parts he can see, and which he knows, by contrasting with other parts, belong to one another. All this is, microscopically, highly educating; indeed, the learner cannot do better than construct an entire follicle, its contents and surroundings, from bits of the parts, then compare his drawing with as entire a structure as his section presents, or with a good diagram of a follicle, such as he will find in numerous works (the 2nd vol. of the 9th ed. of "Quain's Anatomy," page 247, for example).

Now, from what we have written in the previous number and in this, let the observer make out the following:—The medulla, the fibrous substance of the hair, then its epidermic covering. The root part presents its thickening or hair knob, with its denser impregnation of brown pigment granules. He will see at the very bottom of the hair bulb a lighter part (a portion which transmits more light) this is a depression in the bottom of the root, a depression *upwards* really, which admits a papilla. Observe now that we are viewing a papilla, such as we see on the surface of the skin, *sunk* far beneath the level of its fellow papillæ and bearing its *modified* epidermis (the hair), just as its fellow papillæ near the surface bear their layers of *common* epidermis. In this way we see that hair is *modified* epidermis. Not only by tracing the papillæ of the surface and those entering the bottom of the bulbs can we see that the hair is only epidermis in another form, but we see that the epithelium, or epidermis, and the hair have behaved alike in absorbing the dye, they are both brown.

In the present section, we see the little muscles which make the hair "stand on end" to great perfection. They take an oblique direction, and get inserted into the side of the hair follicle, not the hair, just below the sebaceous gland, therefore, when the hair is erected, the whole follicle, with its hair, is moved. These little muscles will puzzle the

observer, if he be not careful to remember, as we have already pointed out in speaking of the follicle and its contents, that the plane of the section may be parallel with *one* entire muscle and shew it from origin to insertion, and other muscles may be cut in any part of their length, even two or three bits of the *same* muscle may be visible. In this case, we find bits of muscle scattered throughout the section, with apparently no use or motive. We can recognise them, however, by their staff shaped nuclei and fibrous appearance and slanting direction, and know how they come to be there.

We see also the dense areolar tissue interlacing all over the upper third of the section, whilst the lower part of the section transmits more light, and is made up of well-defined tissue, subcutaneous tissue, with wider meshwork holding fat.

After observing that every hair root ends in this so-called subcutaneous tissue, and is, therefore, surrounded by it, most excellent training can be got out of the dense *upper third* of the section. Scores of little meaningless (?) bits are scattered through it. The first thing which strikes the attention is the ground work, so to speak, of fibrous connective (areolar) tissue. In this we see bits of muscle of all sizes from entirety to almost nothing: then we see scattered bits of sebaceous gland known by their well-marked polygonal outlines and one well-stained nucleus, and so forth. In other words we must look for the various parts divided in their various planes and expect to find the same structure, the erecting muscle for example, presenting totally different forms according to the *plane of its section*. Much trouble would be saved to learners if they would first study the various aspects of the *same* structure in its different planes: this he might do without a microscope with a bundle of penholders or a lot of straight straws tied together, etc. Cut a common object in different planes and view it. To this knowledge he would have to add the appearance presented by a *part* of an object as by using all powers stronger than an inch, only one shallow plane of an object may be in view. Thus, for rough comparison, take one's breakfast egg, and we see on looking down upon it not only the top, but all the egg, because our naked eye penetrates *all* the planes. If we take an object corresponding in every particular, position also, and view it under the compound microscope, we find, as we lower the tube, the top of the egg appearing as a small round object, with ill-defined outline; and, as we lower the tube, this goes out of sight, and gradually we have a wider and wider, and better defined circle; then this circle again gets less as the tube descends. We see one shallow plane of a thing *only* at any given period, but the part may be so minute that all its planes are visible at once.

When, in searching the upper denser part of our present section, we come upon a bit we cannot make out with our best general searcher, the inch objective, or a power not far from this, we must subject it to a fourth, or higher power still.

Mode of Preparation.

To prepare scalp for sections to be made of it and mounted permanently it is requisite to harden it in spirit, or some other medium. The present section, or rather sections, were hardened in spirit thus:—they were first placed in methylated spirit and common water, half and half, for thirty-six hours, then they were transferred to pure methylated spirit till required. Afterwards, they were embedded in carrot, and sections cut with a razor in the well microtome of Mr. Stirling.

The sections were double stained as follows:—First they were placed in an ammoniacal solution of carmine for a few minutes (5 to 10), made as follows:—

Pure Carmine	...	...	60 grains.
Strong Ammonia	...	...	120 minims.

The carmine is to be dissolved in the ammonia in a test tube, if necessary, by the aid of a gentle heat and then filtered, and the filtrate added to a filtered saturated solution of borax, four ounces. After this they are washed in spirit, then transferred to acidulated spirit ten minutes (meth. sp. 5 parts, hydrochloric acid 1 part). This corrects the ammonia, and clears up any diffuse staining, besides brightening the whole. The acid is, in its turn, to be washed away by placing the sections for an hour in pure meth. spirit. After this they are to be stained in sulphindigotate of soda, two or more ounces, for four or more hours. This solution is thus made:—Take a saturated aqueous solution of sulphindigotate of soda, and add two drops of this to an ounce of methylated spirit when required for use.

The sections are then to be mounted in balsam, after being placed for a few minutes in oil of cloves.

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TRANSVERSE SECTION OF OVARY OF POPPY.

Papaver rhœas (unfertilised)

X 50.

Watson & Son, Lith. 313 Oxford St. Lond.

THE OVARY OF A POPPY.

So far as the mere preservation of the individual life of any particular plant is concerned, the only physiological duties required to be performed by the organism are those every-day operations connected with its respiration and nutrition ; but as it is necessary at an earlier or later period of life to make some provision for the preservation of the species, a special physiological function at this time manifests itself, resulting, under favourable conditions, in the production of offspring, which, strongly inheriting the structural characteristics, modes of growth, constitutional peculiarities, and so forth of the parent, carry forward to another generation the direct line of specific descent. In the higher plants, at all events, this all-important provision is effected by the formation of *embryos* contained within structures known as the seeds, each embryo being the direct outcome of a sexual act ; that is to say, it is produced, or rather its developmental growth is initiated by the coalescence of two masses of protoplasm differing very much from one another physiologically, and derived either from different parts of the same plant or from, what is perhaps more usual, a different plant altogether.

The germ or female element concerned in the process, is a stationary, nucleated mass of naked protoplasm, lying within a comparatively large cell or cavity called the *embryo-sac*, which is a specially developed subepidermal cell of the *ovule*, or incipient seed ; while the sperm or male elements are developed, generally in great numbers, within usually large sacs, or pouches, known as the *anther* cells. Each mass is small, usually more or less rounded, and becomes early invested with a cellulose cell-wall, that eventually differentiates itself into two layers, the inner remaining excessively thin and unmodified, while the outer gradually resolves itself into a surface of cork, specially adapted to act as a protection to the sperm protoplasm, which, sooner or later, is destined to become detached, and to lead, under favourable conditions, a short yet independent existence, removed from the structure within which it was generated. For it should be known, these sperm masses, or *pollen grains*, as they are called, have this special physiological function to perform in the reproductive economy of the plant ; they have to carry to the germ mass the necessary protoplasmic material, or highly specialised sexual food, which, by a process of transfusion, commingles with the female element, and enables it to start a process of growth, and series of developments, which end in the formation of an embryo. After this duty is performed, the activity of the pollen ceases, and it quickly dies.

But in order to understand the exact mode by which the sexual act is effected, let us study as a type the poppy plant, and therein observe how the germ mass receives the fertilising contents produced within the sperm or pollen grains. The ovules or organs containing the germ mass are themselves contained in special vessels the *ovaries*. The ovary is the youngest or central structure of the flower, and in the poppy it is large, oblong or subglobular, surmounted by a conspicuous discoid cap, composed of a number of radiating and cohering *stigmas*, which have surfaces specially developed for the reception of the pollen grains, and which also, at a certain period, secrete a stimulating fluid that tends to encourage the growth of the sperm protoplasm.

Now in plants, like the pea, for example, we have an ovary of a much simpler nature than we find in our type. There, as every one knows, we have an elongated ovary which, during the formation of the seeds, or peas, ripens into a pod. The ovary has a single cavity, and may be looked upon as a leaf which has undergone a special development, whereby its opposite lateral margins have become inflexed and cohering, while from the sutural line of cohesion ovular outgrowths arise at intervals along its entire length. Such a leaf, producing in this way female organs of reproduction, is called a carpel, and the ovule-bearing region is known as the *placenta*. The ovule is usually borne on a stalk—the *funicle*—which is the connecting cord between the parent and its egg, and serves as a channel for the conveyance of food and air to the young embryo during its growth within the embryo-sac.

In tulips and lilies the ovaries are compound structures composed of three such carpels as we find in the pea, but instead of each carpel growing separate from its neighbour, as seen in our larkspurs and anemones, they cohere along their sides, forming a structure which, when cut across, shows that it has an internal arrangement of three cavities, or "cells," with the ovules growing to the acute inner angle of each cavity.

But we may have an ovary where the carpellary leaves are united after a different fashion. In violets and in the mignonette, for example, the ovaries are composed (usually) of three carpels each, yet, as may be easily seen, the structure is only one-celled. Here, however, it is evident that each carpellary leaf has not been folded in the ordinary manner, so as to form a separate cavity for its own ovules prior to the cohesion of the carpels among themselves, as was found in the lilies, but that the adjacent margins of neighbouring and but slightly curved or open carpellary leaves have become united, so as to form one large cavity common to all the carpels; and that, therefore, each of the three somewhat swollen placentæ seen on making a transverse section of the ovary of a pansy is formed by the cohering margins of two similar but individually distinct, carpellary leaves.

In the poppy we have also a multi-carpellary ovary and one, too, where the arrangement of the carpels follows the same general plan found prevailing in the violets and mignonette. The carpels produced in each

flower are, however, more numerous—ten being a common number—and each being just slightly curved and the whole ten being arranged circularly with the opposed sides or margins of neighbouring leaves cohering, as in the violets, we have, as a structural result, the formation of the characteristic oblong or sub-globular ovary of our type. The placentæ, which, as we have seen, are just slightly swollen in the mignonette are, in the poppy developed to an enormous extent. They stretch themselves out into the cavity of the ovary almost, if not quite, to the centre, forming a circle of vertical plates from the broad surfaces, of which the numerous ovules spring, so that in a thin transverse section of the whole organ, as has been made in the accompanying preparation, there is clearly displayed, even to the unaided eye, the outer, or ovary wall of circular outline, with a number (ten or thereabouts) of radiating ovule-bearing bands, each attached by one end to the inner side of the ovary wall, while the other is lying free at the centre, or in the vicinity of the centre of the section. By first examining the section with an ordinary lens, then with a low power of the microscope, and finally a representative portion of the structure, with such a power as that under which the accompanying drawing was made, a comprehensive knowledge of the whole will be gained with a little study. Imbedded in the ovary wall, and opposite the origin of each placenta will be seen a section of a fibro-vascular tissue cord, which has been cut across. These twigs carry upwards the nourishing sap to the growing ovules ; the cords being in direct structural connection below with a similar system, which ramifies throughout the entire poppy plant. The placentæ are principally made up of a rather loose cellular tissue, through which, however, run tiny lateral offshoots from the parietal fibro-vascular cord, and which themselves send off laterally into the funicle of each ovule, a very fine twig to conveniently supply, as beforesaid, the growing embryos with assimilated sap.

The section represents the ovary at a time prior to that of the fertilisation of the ovules, but before describing the act of fertilisation, it will be necessary to learn that the ovule proper is invested in a double coat, everywhere continuous excepting over a spot which is opposite to the sub-epidermal embryo-sac ; here an inconspicuous opening is left, which, in botanical terminology, is known as the *micropyle*. Then, when the female element is ready to be fertilised, the stigma "ripens," that is, it fully extends itself, becomes sticky, or otherwise prepares itself for the reception of the free-moving male element, or pollen. After the pollen reaches the stigma—and the conveying agent may be the wind, insects, or simply gravitation—it immediately begins to grow by sending out a tube, bounded by the extended inner covering which, on first lengthening, bursts through the outer and corky coat of the the grain. The growing tube pushes itself into the substance of the stigma, and continuing its growth downwards, enters the cavity of the ovary. Here it finds an ovule, and, entering it by way of the micropyle, the end of the tube carrying the sperm substance soon approaches the germ mass and fertilises it. After this act the fertilised mass begins to grow, and, fed by

its parent, eventually develops itself into a new individual, while, at the same time, the ovule is being gradually transformed into a seed, and the ovary into a fruit.

The constitutional vigour of the seedling, its own inherent reproductive powers to be afterwards displayed, the number of seeds produced, and such-like important matters bearing upon the plant's vitality, depend largely upon the comparative relationships that exist between the fertilising masses. For example, the relationship must not be so distant that the sexual elements are derived from sources outside the same natural group; while, on the other hand, it has been proved, over and over again, that, in the majority of cases, at all events, self-fertilisation—that is, the transportation of pollen from the anther of one flower to the stigma of the same flower—does not bring about the best progeneratery results. Perfection of the highest order can only be attained when the ovules have been cross-fertilised—that is, when the stigma of one flower is fertilised by pollen derived from the anther of another flower.

Now the form, arrangement, and number of parts, periods of ripening of anthers and stigma, colouring, time of flowering, hours of opening, and other matters in connection with the matured flower, have all more or less important bearings upon the act of fertilisation, and in the majority of plants, tend to obstruct or entirely prevent self-fertilisation, and encourage the more beneficial reproductory act of crossing.

If the fertilisation is to be effected by means of insects, then the flowers have generally showy, easily-seen, and attractive petals, almost always sweet or otherwise scented, while they often also develop nectaries, so placed that to reach them the winged visitors must brush past, push themselves against or walk over some floral organ or organs, so that while they are contentedly sipping the honey, the pollen is being either collected by some part of their hairy bodies, or else being removed from their dust-covered bodies by the sticky surface of a stigma.

On the other hand, if the flowers are wind-fertilised, then the flowers are small, green, and inconspicuous, absolutely scentless, and certainly without honey; and instead of the stigma being more or less knob-like and sticky, it is often either cut up into a tassel-like structure, or else densely hairy, so as to increase the chances of collecting the wind-borne pollen. Compare the showy flowers of the poppy, fuchsia, and honeysuckle, for example, with the inconspicuous flowers of oak, hazel, willow, or oats; while the broad and platform-like, and consolidated stigma of poppy, as compared with the two, long, slender, densely hirsute diverging stigmas of oats, clearly show that these organs are specially adapted for the performance of somewhat different functions, one in fact for insect and the other for wind fertilisation. In the poppy itself each flower produces a great many anthers—and these, borne on rather long filaments, stand erect and close around the radiating stigma. The petals are very large, and, being bright coloured, are hence very attractive to insects. As the flowers do not secrete honey, they are, therefore, only visited by pollen-seeking insects—

which find the broad stigma a most convenient landing stage. The anthers, moreover, burst before the stigma ripens, and the pollen is thus liable to be, at least in greater part, removed before the stigma arrives at its full maturity, leaving it, therefore, greatly dependent for its full complement of pollen upon supplies brought to it by the pollen-dusted bodies of insect visitors. It is likely, however, that many of the extremely numerous ovules may be fertilised by pollen derived from their own flowers, that is self-fertilised. After fertilisation is effected, and embryos are being formed within the embryo-sac, simultaneous changes of a profound nature are also taking place in the structure of the whole ovary. While the now useless petals and stamens are dying the two coats of the enlarging ovule are getting firmer and tougher, the outer becoming more or less coarse, and well suited to act as a protection to the rapidly forming seed. At the same time the ovary walls enlarge, get perhaps, somewhat stouter, and lastly drier, then when the contained seeds are able to live independently, and become detached from the dried up placenta plates, little valves open at the upper end of the seed vessel, immediately below the eve of the persistent stigma and from the pores or openings so formed, the small, almost round, or kidney-shaped, pitted seeds can easily escape at each bending of the stem in the early autumn breeze.

At another time we will attempt a closer study of the anatomy of a seed, and trace the early independent growth of the embryo when thus detached, and removed from the parent, together with the more important physiological facts connected with the phenomenon of germination.

Enough, however, has been said, at present, to explain, in a general way, the structure and use of the flower and to broadly indicate how wind and insects may aid the plant in the dissemination of its pollen enabling it to produce many and healthy seeds, and especially how insects, following their instinctive tastes for bright colours, strong scents, and sweet fluids, are unconsciously developing in plants a diversity of floral forms, encouraging a brilliancy of colouring and increasing their scent-producing and honey-secreting capacity ; and that, therefore, the visits of insects have a decided and very important influence upon the plant's reproductive results, and those flowers that are the greatest favourites with the winged hosts of bees, flies, butterflies, and moths will be the most prolific, and will be the progenitors of new individuals which, inheriting the habits and structural peculiarities of their parents will, still further, perhaps, in their turn, develop those characters which influence the destiny of their race.

Method of Preparation.

The ovaries from which the accompanying preparations were cut were obtained from the just opening poppy flowers gathered in a cornfield at Reading in the month of June last.

After separation from the other organs of the flower they were placed in common methylated spirits for twelve hours, after the lapse of which

time they were removed, embedded in carrot and cut with a sharp razor. A hole of sufficient size may, by the way, be easily cut out in the carrot with the barrel of an ordinary pen.

After cutting, the sections were placed in distilled water for a few minutes, in order to soak out the spirit.

From this, they were conveyed into the staining fluid prepared by adding five drops of Martindale's logwood solution to one ounce of distilled water, and allowed to remain there for about one hour; they were then removed, washed in distilled water, and placed in rectified spirits for two or three hours to remove the water, after which they were taken out and floated on the oil of cloves contained in a large watch glass. So soon as they were observed to sink they were removed and mounted in Canada balsam.

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LONGITUDINAL SECTION OF EMBRYO AT BASE OF WHEAT GRAIN

Stained. Carmine

X 50

A GRAIN OF WHEAT.

The common Wheat plant, of which there are several cultivated varieties, is an erect annual with fibrous roots and a round, unbranched stem, bearing long, narrow, sharp-pointed leaves, parallel-veined and sheathing at their bases. The sheaths, which take the form of split tubes, spring from well developed, ring-like nodes, and run up the hollow, glossy internode giving extra strength to the weak, slender stems, which, in the Autumn time, carry elongated, heavy, terminal heads of densely crowded grains. Each properly developed grain contains an embryonic wheat plant, produced after the manner hitherto described with reference to the common Poppy. But here the organ that becomes detached from the parent, after the embryo has attained its full stage of structural development and physiological independence, is not merely the ripened ovule or seed, but the seed closely invested in the adhering coats of the matured ovary walls, hence, strictly speaking, the grain is, in reality, a *fruit*.

The ovary or incipient grain occupies a central position in the floret, and is a small, sub-globular organ, terminating above in two, long, hairy stigmas. The ovary is a single cavity, and contains a solitary ovule, growing erect from its base, and all but large enough to fill the entire cell. Surrounding the ovary are three stamens, with comparatively large yellow anthers, carried on the ends of long slender filaments. The flowers being either self- or wind-fertilised, can derive no benefit from insect visits, and hence well-formed, conspicuous, perianth leaves are never developed, although rudiments of such organs may be found in the form of two minute scales, lying outside the stamens in the frontal aspect of the little flowers.

Several florets, each protected by two opposite chaffy investing leaves *(glumes)* grow closely upon short slender stalks, while further, these branchlets of associated florets, protected moreover, as a whole, by two outer and larger glumes, are seated upon platform-like notches on opposite sides of the flowering axis, forming, altogether the well-known ear or terminal, compound, spikate, inflorescence of the plant.

After fertilisation is effected, and while the embryo is being formed, a steady transport of nutritive sap sets in towards the seat of reproductive activity. Some portion of the food thus conveyed is immediately used up in the construction of embryo tissue, while other portions go to build up within the ovule, but outside the embryo-sac, a comparatively large mass of cellular tissue, known as the *Endosperm*[1]. In these endosperm cells all the remaining food, that is, the food not required for purposes of present growth, is stored away as a reserve of nourishment for the future wants of the offspring, forming an ever ready, although limited

[1]Synonyms:—*Perisperm*, "*albumen*."

supply of easily available and exactly proportioned food, to be eventually used by the embryo when removed from its parent, and, after a longer or shorter period of rest within the grain, it enters upon the germinating or primary life period of individual existence.

During the enlargement of the ovule, and its conversion into a seed, its outer membranous coat becomes confluent with the inner or lining wall of the also enlarging ovary, and thus it is, that the grain that subsequently falls away from the ear is, as was before stated, a true fruit, or in other words a ripened ovary, containing a fertile seed.

The structure of a fertile wheat grain is the subject of our present study, but, before attempting a microscopical examination of the accompanying preparation, it would be well to procure a few whole grains of any variety of wheat, and, after soaking them in water for twenty-four hours or so, proceed to make a careful preliminary examination of same, so far as can be made out with the naked eye, and a simple or ordinary pocket lens.

The grain is somewhat ovate in form, and plano-convex, and is surmounted by a persistent tuft of short silky hairs; the plain side is traversed by a deep longitudinal furrow; while at the base of the convex side is a pit in which the small embryo is imbedded. Under the dissecting microscope the outer membranous investment (the fruit wall or *pericarp)* may be easily removed, and the true seed skin or testa exposed. The testa, it will be discovered, adheres rather closely to the body of the grain, but over the embryo, where this adhesion is very much weaker, it may be readily detached and pulled aside, revealing the naked plantlet for more easy and clearer observation.

The embryo, as viewed in position, consists of a shortened "collar," produced above into a blunt but rounded cone-shaped bud (the stem-bud or *plumule)* invested in a smooth-surfaced sac of translucent tissue. By carefully opening this sheath, or better still perhaps if it is dissected completely away so as to well expose the enclosed bud, the rudimentary stem-leaves may be nicely displayed as they lie closely crowded together upon the apex of the tiny axis of the plantlet. Springing also from the collar, but in the opposite direction, is a broader structure with two or more short, lateral protuberances—the embryonic root (or *radicle*) with its rudimentary, lateral rootlets, or secondary roots as they are sometimes called by botanists.

Behind these structures, in fact growing from the posterior surface of the collar, is a more or less shield-like structure (the *scutellum*), in the concave or frontal side of which the main portion of the embryo lies; its other or convex side being closely applied to the surface of the before noticed pit, and hence in immediate contact with the endosperm matter of the grain.

The embryo may next be extracted from the pit, and its different parts again examined. By allowing it to slightly dry, an additional structure, a short, median flap of tissue may be seen arising from the collar in front, just at its junction with the radicle.

Having thus obtained a good, general notion of the relative positions of the different structures of the grain, the accompanying preparation may now be examined. It may as well be stated, however, that before the grains were cut they were placed for between twelve and twenty-four hours under conditions favourable to germination ; the sections were then made and afterwards stained with carmine solution.

Using a low power (say one inch obj.) make out the following :—

1.—*The pericarp* or outer coat of grain, detached in many places from seed and often thrown into waves : burst completely at base owing to the slight lengthening of the radicle during its short period of germination : outer layer, large-celled.

2. —*The testa*, or seed coat closely attached to endosperm, but separated from embryo : cells very narrow giving the layer a somewhat fibrous appearance.

3.—*The endosperm (a) outer layer of cells*, cells large, square, and filled with stained protoplasm ; *nuclei*, distinct, intensely stained. *(b) inner extensive tissue of cells*, cells, large polygonal, filled with a granular colourless matter (starch), containing, in addition, protoplasm, which may or may not be stained.

4.—*The embryo*, entirely cellular, made up of
(a) collar, short, broad and central.
(b) radicle, being a downward prolongation of *(a)*, rounded at tip : cells oblong, arranged in longitudinal rows.
(c) coleorhiza, or root-sheath springing from *(a)*, and completely investing, but not adherent to *(b)* excepting at apex.
(d) plumule (a) outer closed sheath (cotyledon of some), *(b)* enclosed bud of rudimentary or stem-leaves, *(e) Scutellum* (cotyledon of others), arising from *(a)*, then spreading upwards, and continued beyond tip of plumule and downwards to same level as tip of radicle, or to its point or junction with the inner side of the coleorhiza : outer layer of cells, narrow, oblong their distal ends touching the endosperm, *(f) short medium flap* arising from anterior face of *(a)*.

Using next the $\frac{1}{4}$-inch obj., re-examine all the structures in the preparation, making out particularly, however, the oval grains of starch in the endosperm cells [3 *(b)* above]. These grains vary very much in size (stated to be from 10-100ths to 6-100ths of an inch), and almost completely filling the thin-walled polygonal cells. Under the increased magnifying power the tissue of the embryo will be also seen in further detail, showing distinctly the cell-wall, protoplasm, and central nucleus of each individual cell.

In addition to the protoplasm there are other nitrogenous but non-living materials contained within the endosperm cells, to which is given, collectively, the general name of *gluten* or

albumen; while also contained within the same cells are various mineral or earthy salts—substances of a highly essential nature in the physiological economy of growing plants, and these are spoken of as the "ash," as they form solid or non-volatile compounds when the grain is burned. According to Prof. Church[1], the following is an average per centage composition of wheat grain.

Composition of wheat grain in 100 parts:—

Water	14·5
Albuminoids	11·0
Starch	69·0
Fat	1·2
Cellulose	2·6
Mineral Matter or Ash	1·7

Respecting the chemical composition of the above constituents, it may may be noted that the albuminoids contain at least five elements—carbon, oxygen, hydrogen, nitrogen, and sulphur; while the starch, fat, and cellulose contain only three—carbon, oxygen, and hydrogen.

Cellulose is a rather complex chemical substance, formed through the activity of plant protoplasm, and is the material out of which the cell-walls of all plants are formed.

Concerning the various ash constituents, the following table, taken from WARINGTON,[2] will give the best idea as to their approximate quality and quantity:—

Average composition of ash stored up in the GRAIN produced by a crop (30 bushels) of ordinary wheat, in lbs. per acre:—

	lbs.
Sulphur	2·7
Potash	9·7
Soda	0·9
Lime	1·0
Magnesia	3·7
Phosphoric Acid	14·3
Chlorine...	0·2
Silica	0·5

From what we have observed, together with a knowledge of the chemical facts put forward in the above tables, we learn that the embryo of the wheat plant is a structure made up entirely of thin-walled cells filled with dense nucleated protoplasm, only differing from one another so far as size, shape and general grouping are concerned; that, lying behind the plantlet and packed away in cells still retaining portion of their protoplasm is a quantity of formative material or readily-available food of a nitrogenous, carbonaceous and mineral nature: and, further, that this food-bearing tissue is closely approached by a broad plate of living cells —a lateral off-shoot of the embryo, a specialised structure, whose functional importance will be presently recognised when we pass on to study the phenomenon of growth as displayed by the germination of the grain.

[1]South Kensington Museum Science Handbooks: FOOD, by A. H. Church, M.A.
[2]The Chemistry of the Farm, by R. Warington, F.C.S.

After the wheat-grain becomes detached from the old or parent plant, the contained embryo may enter upon a temporary period of rest, but so soon as the external conditions are favourable to the performance of the various functions of life, the young plantlet will start upon a regular course of individual existence. These indispensable, or encouraging conditions of seed-growth are, as every one knows, warmth, moisture, and fresh air.

The actual range of temperature within which germination may take place, is, in the case of wheat, and according to Sachs, from about 5°C., to 37° or 38°C. Enjoying a temperature within this favourable range, the embryo will slowly absorb a certain amount of water from the damp earth, in quantity just sufficient to give to the cells a distinct turgidity, as the protoplasm refuses to take up this fluid beyond a particular degree of saturation.

The possible energy of the protoplasm, however, can only be manifested and sustained, through the constant oxidation of complex organic compounds containing carbon. The protoplasm itself, but more often carbonaceous compounds dissolved in the cell sap, are the materials that undergo this process of chemical destruction ; while it is to render oxidation possible that a free and constant supply of fresh or oxygenated air is so important during the life of the plant. The reduction of organic compounds by oxygen, under the influence of living protoplasm, results in the liberation of heat and the production and evolution of carbonic acid gas : while it is this heat, as before stated, that sustains the vital energy manifested by all working cells. The performance of this respiratory function results therefore in perpetual waste of body substance, and unless the embryo has some means of obtaining formative material to counterbalance this unavoidable loss, the plantlet must ultimately and of necessity die. But we have before seen that the endosperm contains a considerable quantity of formative material stored away in its cells, and it is from this source that the now active embryo draws, not only supplies sufficient to counterbalance waste, but enough to enable it to grow. The various food constituents contained within the endosperm cells, exist however, in an insoluble condition, whereas only soluble foods are immediately available for purposes of growth, moreover, the nutrient matters in this case lie in cells at some distance from the embryo, and these can only suffer transportation after their conversion to a state of perfect solution ; therefore before the food can be utilised the embyro must exercise an important influence over the contents of the endosperm tissue, bringing about certain chemical changes in the starch and albumenoids, that will render easy the conveyance of these substances from cell to cell.

From recent researches concerning the real nature of the relationship existing between the cellulose-bounded protoplasm in multicellular plants, it would now seem that the entire bulk of protoplasm enclosed in all the cells of living tissue, is continuous by means of slender threads that pass

through holes of corresponding fineness, left in contiguous cell-walls, and that, as this continuity is especially noticeable in endosperm tissues, we may safely presume the existence of a direct continuity of protoplasm from the embryo into and through all the cells of the endosperm in which the living plasma still persists. At all events, when, under the influence of external favourable conditions, the potential energy of the embryo becomes active, a power, starting from the embryo, is conveyed along the protoplasmic substance into the endosperm or food cells, and works certain changes in the insoluble materials prior to their absorption for purposes of growth. Possibly a secretion or secretions are formed, which, acting chemically upon the different reserve materials, bring about the needful conversion. However, while germination is going on, the starch is gradually resolved into sugar, which of course is highly soluble; the fat is probably reduced through oxidation to starch, and eventually transformed into sugar; while the albumenoids are converted either into asparagin,—a soluble nitrogenous substance, sometimes found naturally in plant saps, notably in young shoots of individuals of the pea family,—or into peptones, highly soluble, proteid bodies which possess in a high degree the property of diffusion.

All these altered, and now soluble, substances are slowly drawn to, and absorbed by, the broad-surfaced scutellum, and passed forward to the embryo proper. The nitrogenous materials supply the protoplasm with the proper formative material required for the building up of its own substance, while the sugar not only supplies material for the construction of cellulose, or cell-wall material, but may also, in part, be oxidised, and hence utilised for the generation of heat.

But transformation of food into tissue can only take place when certain inorganic elements are present, and at the disposal of the active protoplasm; and of these, phosphorus, potash and lime, are certainly the most indispensable during the different stages of germination, and from the figures in the table, quoted above, we know that these substances exist, in comparatively large proportions, in the ash.

While the food materials are being absorbed, the cells of the embryo —and especially those of the radicle—stretch and ultimately grow by bipartition of cell at the apices. The rudimentary rootlet-protuberances soon burst the close apex of the more slowly growing coleorhiza, and emerge as slender, colourless threads, as shown in Plate 6, Fig. 1. The plumule, at this time, has also pushed itself forward, the sac-like conical leaf keeping pace, in its growth, with the enclosed rudimentary, but developing foliage, binding the separate leaves well together, and leading them safely upwards, through the particles of earth, into the outer air and quickening sunlight.

As the embryo grows, the epidermal cells of its elongating rootlets throw out a great number of hairs—as may be seen in Fig. 3—while the oldest leaf of the now surface-reached plumule bursts the apex of the

GERMINATION OF WHEAT.

Weidner & Son Lith. 30 & 32 Charles St Boston

ensheathing leaf,—as is also shown in the same Fig.—and rapidly lengthens itself by the individual enlargement of its constituent cells.

About this time the store of nourishment contained within the endosperm cells will be entirely exhausted, or nearly so, thus throwing the young plant completely upon its own resources, and hence formative materials, similar to those upon which the sprouting embryo was nursed, must now be manufactured by the plant itself, to enable it to carry on all subsequent growth and development.

The roothairs, as they grow, become closely adherent to the particles of soil, so much so, indeed, that if the plantlet be carefully uprooted, and then rather roughly shaken, the inorganic particles will be seen to still firmly cling to the crop of hairs (Fig. 4), and these tender structures will even more readily break away from the parent rootlets, than part company with the tiny fragments of attached earth. If these roothairs be carefully washed, and examined under a proper magnifying power, this pseudo union may be easily observed. In Fig. 5, is represented the tips of three roothairs, of different ages, which illustrate the gradual metamorphosis of these important organs of absorption. It is obvious that in a soil, where there is a suitable amount of moisture present, all the readily soluble earth-salts will be easily taken up by these hairs, in the ordinary osmotic inpassage of water, that—subject to a favourable temperature—is constantly taking place, from the surrounding soil; while over and beyond this, the protoplasm contained in the root-hairs, exercises, by virtue, perhaps, of an acid secretion, a solvent power upon the more difficult soluble salts (phosphates for example), and thus increases, considerably, the percentage of valuable earthy matter, daily collected by the plant.

The now unfolded leaf performs several very important functions in the physiological economy of the plant. It manufactures starch, for example, it takes in atmospheric air, and permits the escape of useless gases, and it frees the plant of superfluous water by a process of transpiration. In structure, the leaf is composed of several layers of cell-surfaces, interrupted by the so-called veins, the individual cells in some of the layers being so loosely arranged that intercellular spaces are very common in this part of the tissue; while all of these cells contain active protoplasm, in which green-coloured bodies are embedded. A layer of flatter, more closely-fitting, colourless cells, which have entirely lost their protoplasmic contents, form a surface boundary layer, above and below. Some of the cells in both of these epidermal layers, instead of passing into ordinary epidermal cells, retain their protoplasm, and develop into the guard cells of special openings or stomata. These cells are semi-lunar in shape, and are always distributed in pairs, and arranged so that their concave faces are opposite to one another, thus forming a minute oval passage leading directly into the intercellular spaces of the leaf. The green-coloured bodies above referred to, are dense grains of protoplasm, which, in presence of ever so minute quantities of iron, together with normal exposure to sun-

light, become so coloured with a pigment known as chlorophyll. In these green cells of the leaf, starch is manufactured, and the raw materials at the disposal of the cells are carbonic acid gas (CO_2) obtained from the air and water (H_2O) absorbed from the soil. These, under the influence of sun-exposed, chlorophyll-bearing protoplasm, are, at all events, partially destroyed, and their elements re-arranged to form the ternary organic compound, starch ($C_6H_{10}O_5$). This starch may remain in the cells of the leaf during the hours of sunlight, but at night it is slowly converted into sugar, and carried backward to feed the protoplasm in the darker regions of the plant's body; that is, not only may sugar be used by the active protoplasm for purposes of oxidation and for the construction of new cell-walls or the strengthening of already-formed tissue by the elaboration and deposition of fresh cellulose material upon old walls, but it may, in part, be used for the formation of albumenoids, the necessary nitrogen and sulphur being obtained from simple nitrogenous and sulphur-containing compounds dissolved in the sap. All the water, as well as the whole of the various chemical ingredients required by the working cells of the plant, not only for the maintenance of their individual vitality, but also for the construction of new tissue elements, are, with the exception of carbon, obtained from the soil through the active agency of the slender root-hairs: hence the importance of these organs in the plant's physiological economy.

EXPLANATION OF PLATE 6.

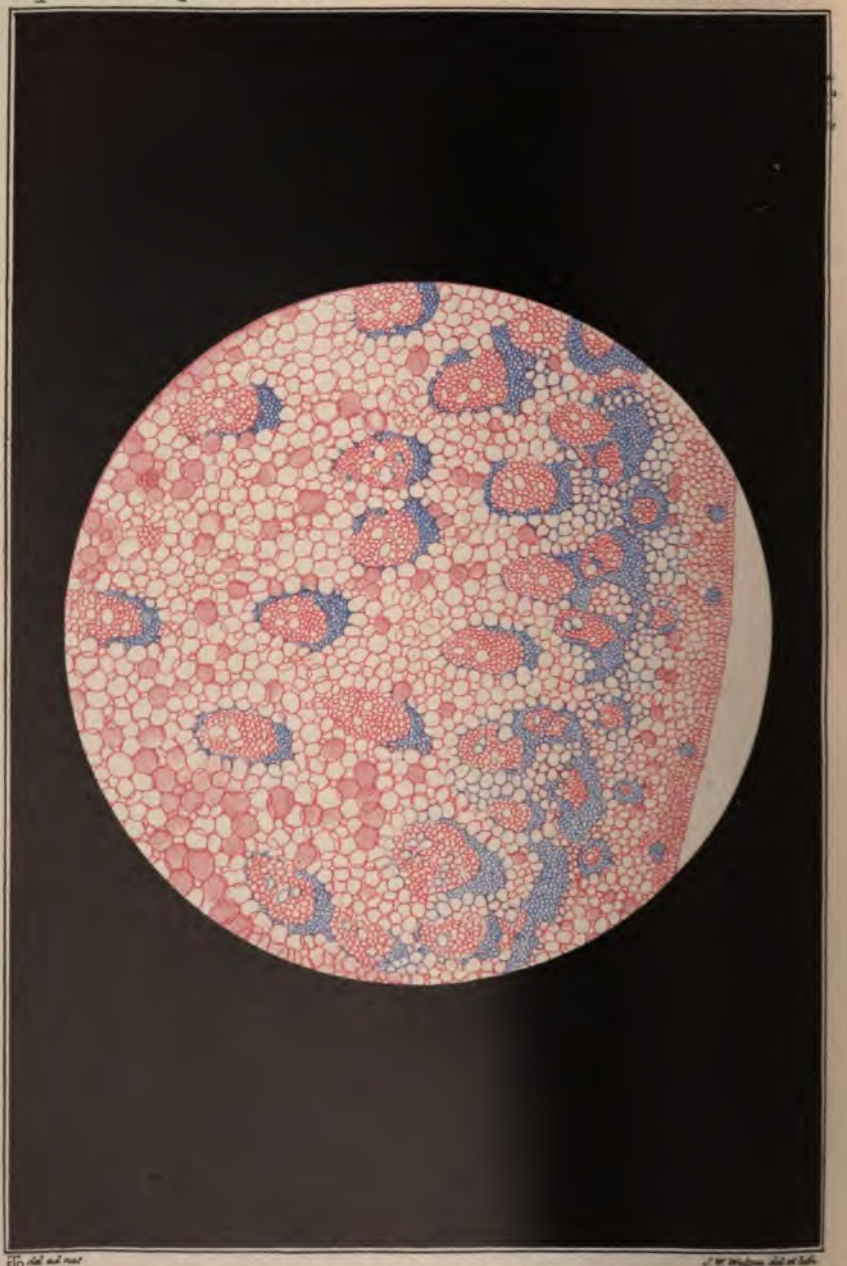
Fig. 1.—Germinating Embryo of Wheat; the leaves of the plumule still included in the sheath leaf, while the rootlets have sometime burst the coleorhiza (after Bocquillon and copied from Brown's *Manual of Botany*).

Fig. 2.—Longitudinal section of grain in stage represented in 1. The Scutellum lying against substance of Endosperm, and the internal structure of the bud stem, are particularly displayed. (Copied from Brown, op. cit., .)

Fig. 3.—Further stage in germination—the oldest plumule leaf has burst the sheath, while the rootlets have developed roothairs. (Copied from Hooker's *Primer of Botany*.)

Fig. 4.—An uprooted plantlet showing close adherence of soil to roothairs. (After Sachs, copied from his *Handbuch* der Experimental Physiologie der Pflauzen.)

Fig. 5.—Tips of roothairs (strongly magnified) in three different stages, showing more closely the intimate connection between the particles of earth and the walls of the hair (Sach; op. cit., .)



TRANSVERSE SECTION OF STEM OF BULRUSH (TYPHA)

Double stained.

X 75

THE COMMON BULRUSH.

The Bulrush is a well-known, reed-like plant, with tall, stately, poker-like flower-heads, found by margins of lakes, sides of streams, in marshes, water-ditches, and such-like situations; and freely distributed throughout the temperate and tropical regions of the whole world, especially, however, affecting the Northern hemisphere. It has been selected to illustrate the general structure of a flowering-plant, after it has attained the age of full maturity.

The erect, leaf-bearing structure is, in reality, a branch, and springs from a prostrate stem, imbedded in the mud-covered bottom of the ditch. This creeping, thickish stem, known as a *rhizome*, produces from its sides a number of slender, spreading rootlets, that not only help to keep the plant firmly fixed in position, but are also functionally concerned in the intaking of food.

While the aërial portions are of mere annual duration, the rhizome is perennial, throwing up year after year, in each returning Spring, the erect, herbaceous structures that will complete their growth long even before the end of Autumn. The rhizome keeps its younger or apical end always in a histologically primitive condition—that is, its tissue is made up entirely of cells, which are rich in protoplasm, and still retain the power of self-division; such a tissue is known as *meristem*. New cells are being continually formed in this region during the period of active growth; those that are pushed back, or left behind by the slowly advancing growing point produce by different developmental lines of growth, all the various members, organs, and systems of tissue found in the mature Bulrush plant.

The aërial branch arises as a tiny, lateral mass of meristem cells, upon the surface of the cone of growth, at a little distance behind the extreme apex. This luxuriant assemblage of cells becomes a secondary cone of growth; it grows slowly, perhaps, at first, but in time produces a series of lateral protuberances that are the rudiments of leaves. These primitive leaves, growing more rapidly than the apex of the axis that produces

them, and the cells of the under sides growing a trifle faster than those of the upper, tend to curve themselves over the still active apex, producing a structure known as a *bud*. The older or first-formed leaves, that is, those lying on the outside of the group, are not destined to become green or foliage leaves of the plant; they become somewhat different in form, coarser in texture, and less succulent—in fact, they act functionally as a protection to the younger and more tender leaves within. The youngest, or last-formed lateral processes of all, from this secondary axis, develop into rudimentary, pollen-bearing and ovule-producing organs, after the production of which the apical region becomes exhausted, and at once ceases to grow by division of cells.

The bud structure now described is just completed in the late summer of the year previous to its appearance above the surface of the mud, ready in the next succeeding spring to shoot upwards with a rapid growth principally by the combined result of mere individual elongation of cell. To feed these buds, the Bulrush plant stores away every year within the cells of its rhizome a considerable quantity of starch and other necessary food stuffs, upon which the young shoots are nursed until they can assimilate formative materials for themselves.

The foliage leaves of these freely-growing shoots are long, narrow, and linear; they are provided with well-developed sheaths that completely clasp the stem and contribute in no small degree to its strength. The poker-like head of minute, densely packed flowers is carried some distance beyond the insertion of the uppermost leaf by the growth of a considerable length of bare, rigid stem. The flowers are 1-sexual, but both male and female are upon the same individual. The staminate flowers occupy the higher position on the axis, while the pistillate florets form the lower, extremely dense, velvet-like portion of the inflorescence.

If a leaf be broken across it will be seen that the veins are formed of long whitish threads, which may be easily traced backwards into the sheath, and from thence into the stem. Here, the threads run with a gradual, downward sweep towards the centre, approaching which they bend and make their way with a still more gradual course towards the periphery, eventually thinning themselves off, after following a line sometime parallel with the surface of the stem. It is this crowding, as it were, of the attenuated end portions of the tough fibrous looking threads towards the circumference, that gives to the outer portion of the stem its characteristic firmness, enabling it to uphold itself erect, and support the heavy, cylindrical head of densely-packed florets.

The further structure of the stem may be conveniently made out by an examination of transverse and longitudinal sections, such as are distributed with this description. It may be seen from the transverse section that in the stem there is an outer boundary layer of cells—the *epidermis*—enclosing a mass of ground tissue, or cellular matrix, in which the cut ends of the threads before noticed are seen to be embedded.

From the longitudinal section we learn that these isolated strings are made up of narrow, elongated, much thickened cells or fibres, and wide continuous tubes, formed by the coalescence of longitudinally superimposed cells, presenting banded or spiral thickenings upon the inner surfaces of their walls. They are known as *fibro-vascular* bundles.

These specialised, histological structures arose in a sub-circumferential region of cells (a ring, in transverse section) that, retaining for a limited period their primitive power of growth by sub-division, formed a secondary meristem region, in which new tissue systems were readily initiated. The fibro-vascular bundles are formed in this tissue within certain prescribed boundaries. Some of the cells grow individually as it were; they attain their mature form, lose their protoplasm, and, still retaining their cell identity, become permanent; while others may be described as being decidedly social in their growth, seeing that from the first they are arranged in longitudinal rows, the individual cells of which become in time completely fused, while their separating end walls becoming eventually ruptured and absorbed, produce the long tubes or vessels so clearly observable in the longitudinal section.

As new fibro-vascular bundles were formed in the region of secondary meristem, the older bundles were simply pushed towards the centre, until, the activity of the meristem ceasing, no further additions were made to their number. The cells of the now spent meristem become at once the seat of special individual growth and modification. Their walls become first very much thickened and afterwards lignified, producing a tissue system of considerable strength, and well shown in the transverse section, just a little beneath the Epidermis. It has taken up the green stain, and is known as the *thickening ring.*

It is not our object in this series to describe minutely and technically the different structures to be observed in any particular preparation, but we may direct attention to the general arrangement of parts in one of the fibro-vascular bundles. To the inner side of the bundle is a group of woody fibres, and to the outer side a conspicuous patch of liber cells, with very much thickened walls, intensely stained with the green dye. Lying between these are cells and vessels. Some of the cells are thin walled, others thick. All the vessels have sculptured walls, and, comparatively speaking, are rather wide, two or three of them indeed being extraordinarily so. These bundles are functionally concerned in the conveyance of water from root to leaf, and in the distribution of sap and oxygenated air throughout the entire plant.

E.T. del. et nat.

J.W. Madsen, Lith. et Lith.

T.S. ILEUM OF CAT INJECTED
X 50

Watson & Son, Lith. 313 Chancery Lane, Lond.

THE INTESTINE

[illegible]

[illegible]

[illegible]

[illegible]

l.m., layer of [illegible]
s.m., sub-mucosa; [illegible]
panying preparation[illegible]
but only the blood vessels.

It will hardly be necessary [illegible]
animals provided with [illegible]
it (Lat., verto, I turn—[illegible]
plicity, consists of a [illegible]
This tube, [illegible]
is called the Alimentary [illegible]
almost all cases, it is [illegible]
the body cavity, and [illegible]
outside, so that anything [illegible]
side the body.

In no vertebrate is the [illegible]
the same shape throughout, [illegible]
some (the pipe fishes for instance) [illegible]
dition. Some parts are [illegible]
class—mammalia (Lat., mamma, the udder)—
the cat belong. [illegible]
the mouth opens [illegible]
in the name "mouth," though [illegible]
opening, and the cavity [illegible]
mouth). This leads into a [illegible] (Gr., *oro*, *oros*, I stand early; *oryx*, *pharugos*, to eat; **pharugx**, gullet), which [illegible] the gullet or œsophagus (Gr., *oiso*, *oisō*, I shall carry; *oryx*, *phagein*, to eat)—which suddenly expands again and attains its greatest diameter in a sac-like part called the stomach, after which it as suddenly contracts again to form a long tube—the intestine, or gut (Lat., intus, within),

which again expands somewhat for a considerable length near its termination, and this wider portion is called the large intestine to distinguish it from the narrower, small intestine. The small intestine is much longer than the abdomen in which it lies (in the cat it is about 3½ feet long), and as a natural consequence it becomes very greatly convoluted. It is covered by a membrane which also invests the other abdominal organs and lines the abdominal walls. At the back of the intestine the two halves of this membrane run together to form a single sheet—the mesentery (Gr., μέσος, mesos, middle; ἔντερον, enteron, intestine), by which the folds of the intestine are secured, and the whole suspended in the abdominal cavity. The mesentery is also the channel by which the various vessels and nerves of the intestine are carried. It is called a serous membrane, because it secretes a fluid called serum, which keeps it constantly moist.

The small intestine is without much show of reason, except convenience of description, usually divided into three regions. The shortest of these that which immediately succeeds the stomach is called the duodenum (Lat., duodeni, twelve) from the circumstance of its being about twelve finger breadths long in man. Of the remaining portion of the small intestine the first two-fifths are called the jejunum (Lat., jejunus, empty) because it is usually found empty in post-mortem examinations; and the last three-fifths constitute the Ileum (Gr., εἰλέω, eileo, I roll up) with which we are more immediately concerned.

The walls of the alimentary tube from the œsophagus downwards are composed of three principal sets of structural elements, typically represented in the ileum, each having a distinct function to fulfil. Commencing at the outside beneath the serous investment, which itself is sometimes enumerated as one of the coats of the intestine, we have two layers of muscular tissue which from the fact, patent to everyone, of their being beyond the control of the will, are called involuntary, and from the simple elongated-cellular nature of their fibres and the absence of either longitudinal or transverse striation, such as characterises all voluntary muscles, are called unstriped or non-striated. The outer coat, which is much thinner than the inner, has its fibres arranged in the direction of the length of the intestine, and the inner coat has them disposed concentrically with it, or circularly, like so many elastic bands, except that no individual fibre cell is long enough to go all round. It is by the contractions of these coats in regular and successive order from above downwards that the food is gradually forced along the alimentary canal and brought under the influence of its various digestive and absorptive organs. Internal to these muscular layers, which constitute one of the chief sets of structures above referred to, comes an areolar (Lat., areola, a little space), fibrous or connective layer of loose and open structure, in which the large and numerous vessels can ramify and by which the muscular layer is connected with the third principal coat chiefly cellular in structure, called the mucous coat (Lat.,

mucus, the nasal secretion). This mucous coat is composed in the main of an enormous number of simple (intestine) or branched (stomach) tubes placed side by side perpendicular to the surface on which they open, and lined with a layer of cells, each one of which is a morphological unit or physiological working man charged with the duty of manufacturing from raw materials supplied by the blood some part of the digestive fluids. Each tube opens on the free inner surface of the intestine and forms the channel by which the little droplets, secreted by each cell, find their way to unite with those discharged from neighbouring tubes. When it is borne in mind that the total number of these tubes in the small intestine of the cat amounts to many hundreds of thousands, there will be no difficulty in crediting them with the secretion of a considerable quantity of fluid. Besides these simple tubular glands, others of larger size and more complex structure occur, but they must be dismissed with this passing reference. The gland cells, which are spherical or polygonal in the deeper parts of the glands, assume by imperceptible gradations an elongated or conical form as they approach the surface, on reaching which they become continuous with a complete layer of columnar epithelium, which lines almost the whole canal.

Distributed all over the inner surface of the small intestine is an immense number of little conical, cylindrical, or finger-like extensions of the mucous coat called villi (Lat., villus, nap of cloth). So numerous are they, that if a piece of the small intestine of a cat be opened, washed, and examined under water, with the aid of a lens, they give it almost the appearance of velvet pile. The centre of each villus is occupied by blood and lymphatic vessels supported by fibrous tissue, and its surface is covered with columnar epithelium continuous with that of the general surface.

The whole of the alimentary canal is abundantly supplied with blood vessels, which differ in the details of their distribution in different regions, in accordance with the special structure and special requirements of each part. To the small intestine the blood is brought almost directly from the heart by a large artery (the superior mesenteric) which traversing the mesentery, there divides into numerous smaller arteries for the supply of all parts of the small intestine, on the outside of which they ramify immediately beneath its serous investment, and then penetrate its muscular coats, to which they distribute a few small twigs as they pass, but do not branch much until they reach the submucous or areolar coat. Here they break up freely, and form a dense network of minute arteries, which send some branches down to the muscular coats, where they immediately break up into capillaries; and others up into the mucosa, round the tubular glands of which they form a close capillary investment. Other minute arterioles enter the villi and form capillary loops which, again uniting, form the ultimate veinlets by which the blood is returned from the villi. These veinlets unite with others in the mucous coat to form larger veins, which in the submucosa become yet larger by the accession of branches from the muscular layers, and

finally make their exit by piercing the muscular coats, from which in passing they receive a few more minute branches. These comparatively large veins usually run alongside the corresponding arteries from which they may be distinguished in injected preparations by their larger size, and uniting with their fellows on the outside of the intestine beneath the serous coat they form a few main trunks which all run together to form the large superior mesenteric vein. This vessel carries its blood into a large vein (the vena porta) which conveys it to the liver, through the capillaries of which it has to pass before it is finally discharged into the main venous trunk of the body.

Besides the blood vessels, there is another set of vessels somewhat resembling small veins with excessively thin walls, abundantly present in the intestines. These are called lymphatics (Lat., lympha, water), from their colourless contents. They commence in the centre of the villi as straight or looped tubes with closed ends, and of greater calibre than the blood capillaries by which they are surrounded. In the submucosa they form a dense plexus, the main branches of which piercing the muscular coats, find their way to the mesentery, which they traverse, passing in their way through numerous glandular bodies—the lymphatic glands—and then discharge their contents into a main lymphatic vessel—the thoracic duct—which communicates directly with the central venous system. For the demonstration of the lymphatics, a special kind of treatment is necessary, and the reader must not expect to see them in the accompanying preparation.

Having studied the machinery of the alimentary canal, let us now see what work it is destined to perform for the great commonwealth of the body. We know that its possessor is constantly in action, for even during sleep the mechanisms of circulation and respiration—to say nothing of digestion—must be kept going, and regulated by the activity of the nervous system, and we know also that wear and tear are the necessary consequences of the working of any machine, lifeless or living. There is a constant loss of matter, which must be as constantly replaced if this state of activity is to be maintained for any length of time. The cat's tenure of life, or the maintenance of its functional activity is dependent also on another condition—its body must be maintained at a constant temperature, usually much higher than its surroundings, and for the production of the necessary amount of heat, fuel must be supplied. During early life too, the animal has to do something more than maintain a constant temperature and a diurnal balance of matter, it has to increase in size. For all these purposes and others, food must be introduced from the outside world, and the duty of the alimentary canal is to reduce this food to a state in which it can be absorbed and to provide facilities for its absorption. The cat's food consists partly of fluids, partly of solids, but before the solid parts can be absorbed they must be dissolved or digested, a process which is accomplished by certain chemical solvents, manufactured by the various glands of the alimentary canal. As a first

step to this end, the solid food is reduced by a mechanical process of attrition or mastication to a state of fine division in order that the various digestive fluids may have free access to all parts of its substance; it must then be carried along to the different regions of the canal, each one of which has a specific action on certain of its constituents only; for instance, in the buccal cavity a class of food stuffs well represented by starch are affected, the stomach is specially concerned with muscular tissue, and the small intestine with fat. The transporting power resides, as we have seen, in the muscular tunics of the alimentary canal.

It is in the stomach that provision is first made for absorption, for it is there the food is first rendered soluble to any considerable extent (in carnivors, at all events); but the small intestine is the organ par-excellence of absorption, and the means by which it is specially adapted to the performance of this function are twofold. In the first place it is made to present in a very limited space as large an absorptive surface as possible over which the chyle (Gr., χυλός, chulos, juice), as the liquid product of digestion is called, must flow, a condition which is secured by its great length, and is further enormously increased by the numerous villi with which it is studded, and in the second its abundant blood and lymphatic vessels are brought into the closest possible relation to the chyle, as we see in the villi, between the interior of whose vessels and the chyle by which they are surrounded on all sides nothing intervenes but the single layer of epithelium cells, a few straggling connective tissue and muscular fibres, and the exceedingly delicate walls of the vessels themselves. Indeed, the epithelium cells should properly be excluded from this category of passive impedimenta, for they are really active agents of transmission, and, moreover, probably exercise a selective power, and so facilitate instead of opposing the process of absorption. The more soluble and diffusible portions of the chyle find their way by simple osmosis directly into the blood vessels, but the fat, which is not really dissolved, but only finely divided (emulsified) and held in suspension, as in milk, passes into the lymphatics, and so enters the blood-vascular system by a roundabout course. Whether this is due to a selective power exercised by the lymphatics themselves, or the epithelium cells, or by other means, is at present uncertain.

Having aided the digestion of the food, transmitted the chyle into the blood and lymphatic vessels, and passed on the insoluble or innutritious residue of the food into the large intestine, whence it can be expelled from the body, the work of the small intestine is accomplished. At this point the circulatory system takes up the work, and the absorbed constituents of the chyle, after passing through certain elaborating or metabolizing (Gr., μεταβολή, metabole, change) glands, chief of which are the liver and the lymphatic glands, in which they undergo important changes, are carried to the heart, from whence they are distributed as constituents of the blood to all parts of the body for constructive, restorative, and heating purposes.

HEAD OF TIPULA

X 40

THE CRANE FLY *(Tipula Oleracea.)*

DESCRIPTION OF PLATE.

n neck, *e* eye, *a* antenna, *mp* maxillary palp, *l* lobe of labium.

The common Crane Fly or Daddy Long Legs, or as it is called in some parts of the country, Harry or Peter Long Legs, is a very well-known British insect. Its larva or grub indeed enjoys quite an unenviable notoriety, for the reason that the little brown, legless and worm-like creature no sooner emerges from the egg among the roots of a grass crop, or garden lawn, where it has been deposited by a careful parent. than it applies itself with an energy undisturbed by other cares to the one absorbing object of its life at this period. Its destiny is to eat, and eat it must assuredly does, with a degree of perseverance worthy of a better cause. So great is its voracity that the roots of the grasses, and other plants where Tipula-larvæ abound, are so completely eaten away by them that it has been found possible, over large areas, to roll up the withered turf as easily as if a turf cutter had been under it. Naturally, under these circumstances, the grub increases in size, and after a few months it passes into the quiescent state of a pupa, and then eats no more, but undergoes a series of metamorphoses, resulting in the development of wings, antennæ, or feelers, legs, and other organs, external and internal, and there emerges from the ground some fine autumn evening the perfect dipterous (Gr. dis, twice; pteron, wing) insect which is the subject of the present study.

In considering the anatomy of the crane fly, the first point to strike the observer is the division of its body into three distinct regions—head, thorax or chest, and abdomen. The abdomen is easily seen to be made up of a series of annular segments, and a study of its development, and of its relation to allied animals, shows that the thorax and head are constructed on the same type, though in consequence of developmental changes bringing about the fusion of originally separate parts and the suppression of others, it is very difficult to make this out in the head.

The abdomen is devoid of appendages, except in the female, an organ (ovipositor) for the deposition of its eggs; but each of the three segments of the thorax bears a pair of enormously long legs. The middle segment of the thorax also carries a pair of membranous wings, and the third segment a pair of filamentous bodies with knobbed extremities (halteres or balancers) of very doubtful utility; and believed by some to be the abortive representatives of the second pair of wings possessed by most insects. The appendages of the head must be left for more detailed consideration presently. The integument of the body and appendages is composed of a substance called chitin, resembling horn in its physical properties and forming a resisting outer case or exoskeleton, but hard internal parts are absent.

The internal organs of Tipula comprise a heart in the shape of a long contractile tube on the back or dorsal side of the body, an alimentary canal with accessory salivary and hepatic glands, and a double chain of nervous ganglia placed on the lower or ventral side of the body, with one large ganglion or assemblage of ganglia in the head above the œsophagus, and answering in function to the brain of higher animals. Respiration is carried out by branched tubes (tracheæ) which carry air all over the body and limbs, and whose main branches communicate with a pair of openings (spiracles) in each segment of the body, except those of the head whose tracheæ spring from spiracles at the sides of the neck. The collapse of the tracheæ by accidental pressure is prevented by a spiral thickening of their chitinous lining.

The head of Tipula is elongated in a vertical direction, as shown in the plate, which represents a front presentation as it is seen in the accompanying preparation, but it must be pointed out that the antennæ and maxillary palpi are necessarily constrained by the cover glass to assume unnatural positions. In life the antennæ would be extended in the line of sight towards the observer—that is at right angles to the plane of the paper, and the maxillary palpi would be carried backwards under the head. The first objects to claim attention are the eyes. Examined under a 1-inch objective in a strong reflected light their surfaces will present a hexagonal (sometimes square) areolation, like those facetted lenses or spy glasses which in our childhood days amused and puzzled us with their multifold images. The further study of the insect eye must be made by means of vertical sections. It will then be seen that each hexagonal area or corneule, is a doubly convex transparent body, which must therefore act as a lens, and there is reason to believe that, in some insects at all events, each corneule is composed of two halves of different density, whereby the production of false colour may be avoided. Behind the corneule, and separated from it by a ring of pigment, which answers the purpose of an iris, in limiting the path of the rays to the central portion, is a transparent cone with convex ends, placed with its base outwards or next the

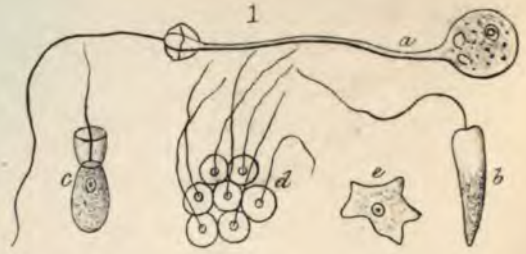
iris and its apex in connection with a single fibre of the optic nerve. All these structures go to make up a single eyelet or ocellite, and each is separated from its neighbour by a layer of dark pigment. The whole assemblage of a thousand or so ocellites, arranged side by side, with their apices converging to one point and their bases forming the external corneal surface, go to make up the single compound eye. The hexagonal form is the result of mutual pressure. Now, though it has been proved that each ocellite is capable of forming an image at the point of junction with the optic nerve, it does not at all follow that each is the analogue of the vertebrate eye, and that there are formed as many complete images of its surroundings as there are facets in the eye; for in consequence of the pigment separating cone from cone, each single nerve fibre can be impressed only by the light received through the single ocellite with which it is connected, and as the field of view of each is very small, and they are all turned in different directions, a different view is presented to each, though there would be some such overlap as in a pair of stereoscopic photographs. And then who could credit a single nerve fibre with the fearfully complex function of transmitting the impression of a complete image? We must therefore conclude that but a single picture is formed by the eye; that picture may be regarded as a mosaic composed of as many points or pieces as there are corneules in the eye. It must necessarily be imperfect, not only by reason of the limited number of points of which the image is composed, but because each nerve-ending is impressed not only with the light from a single point, but from all points—and there may be many—included in its field of view, however small that may be.

The next organs to be noticed are the antennæ. Each will be seen to consist of a large basal joint, followed by a short cup-shaped piece, and then a series of larger but gradually decreasing joints, the whole organ acquiring a beautiful form by the whorl of stiff, bristle-like hairs around the base of each joint. It appears certain that the antennæ are tactile organs, and that they serve in some way for intercommunication between individuals, and there appear reasons for believing that in some insects they have also an auditory function. Below the antennæ, and even exceeding them in length, will be seen a pair of filaments, each consisting of four joints; the basal joint springs from the back of the face, and the terminal one enormously exceeds the others in length, and all are plentifully besprinkled with short, stiff hairs. There are the maxillary palpi. They probably perform a tactile function.

The mouth is situated below and behind, and from it springs a short fleshy trunk, tumid and bilobed at its free end. This organ is the so-called tongue or proboscis. In the accompanying preparation its lobes, which are covered with bristly hairs, will be seen projecting below the face, and in the interior of each will be seen two large tubes with numerous branches; their chitinous lining is thickened in a manner that will at

once recall the idea of tracheæ, which these tubes, as a matter of fact, really are, though they have been appropriated to an entirely different purpose from that of respiration. They are channels through which the liquid food of the fly is sucked up into the mouth.

We shall arrive at a more correct understanding of the nature of the parts of the mouth (trophi), which we have examined in Tipula, if we examine the corresponding parts in an insect in which they are more normal. Let us take the common cockroach. Here the mouth is bounded before by a median horny plate, the labrum or upper lip, and behind by another median plate, the labium or lower lip, with soft foliaceous appendages, the labial palpi. At the sides of the mouth are two pairs of moveable jaws, an anterior pair of strong toothed cutting plates, without appendages—mandibles, and a posterior pair more foliaceous the maxillæ, each provided with a large external palp. The trophi, therefore, consist of a median labrum, a pair of mandibles, a pair of maxillæ, and finally a labium, which really represents a second pair of maxillæ, united by their basal parts. In Tipula the labrum and mandibles are represented by three minute pointed styles or setae contained in a groove on the upper side of the proboscis, and invisible without dissection. The first pair of maxillæ are represented by their palpi which have undergone no abortion, and the labium or confluent second pair of maxillæ, forms the fleshy proboscis.



TYPES OF SPONGE STRUCTURE

SPONGE.

DESCRIPTION OF PLATE.

1. *a b and c* Flagellate sarcoids, *a* and *c* with collar, *d* group of flagellate sarcoids seen in plan, *e* Amoebiform sarcoid.
2. Semidiagrammatic section of horny sponge showing the oscula and ciliated chambers. The arrows indicate the direction of the currents.
3. Section of horny sponge—Chalina oculata with spicules in situ.
4. Various forms of sponge spicules.

The soft, tough, porous material which lends its name to this article is perfectly familiar to everyone, and most persons have a vague idea that it is a production of the animal or vegetable kingdom, the general impression being that it is due to an insect similar to the creature credited by popular fancy with the formation of coral. Indeed it is really wonderful how comprehensive the term "insect" is in the mind of the non-biological public. Just as any physical phenomenon which is not understood is unhesitatingly ascribed to electricity, so any animal smaller than a mouse, unless it be a fish, is an insect. But even this idea of the origin of sponges, vague and erroneous as it is, marks an advance on a previous state, for it is not so very long since even naturalists of eminence, who had studied the sponge, were unable to agree as to its animal nature at all, many stoutly maintaining for it a vegetable origin. So for fifty years the sponge remained in purgatory unable to find a permanent place in either kingdom. Nor can the biologists of the present generation plume themselves very highly on their comprehension of the nature and affinities of this animal or colony of animals, which still remains in this year of grace 1884 as great an "anomaly" as "the Conservative working man."

In taking up the study of this "form of life," we must be prepared to descend very low indeed in the scale of life—almost to the very bottom of the animal kingdom—to those realms where heads, limbs, bones, muscles, mouths, vessels, nerves, lungs, and organs of sense, or of anything else for that matter, are left far behind, and the animal or zooid, if it possesses what may without flattery be called a *shape*, may be congratulated on its superiority over some of its near relations.

The material known in commerce as sponge is obtained by divers from the sea-bottom in the neighbourhood of the Greek Archipelago, the Bahamas, and other parts of the world, but it is not the whole animal but only a supporting skeleton from which all the living matter has been removed by washing, squeezing, and bleaching in the sun. If a piece of this sponge be examined with the naked eye there will be seen numerous large, more or less circular openings leading into canals which branch and penetrate the sponge in all parts and freely communicate with one another. A simple lens of high power will show that the substance of this skeleton is composed of an open feltwork of curling and branching fibres of a horny substance called *keratode* (Gr. *keras*, horn; *eidos*, form). In a living sponge this cannot be made out, for the skeleton is then covered with a slimy material and only the larger openings are then visible. Sections of the living sponge in any direction would show that this same slimy substance pervaded the whole interior, covering all the fibres and lamellæ and leaving only a series of narrow, branched canals, the smaller branches of which communicated with a number of microscopic openings called "pores" in the outside of this gelatinous mass.

If a healthy sponge be examined in some of its native water, to which some finely-divided solid substance—say carmine—has been added, it may be observed that currents of water are constantly flowing into these pores, while other currents are streaming away from the larger apertures, called *oscula* (Lat. dim. of *os*, mouth). It is thus evident that there is a constant circulation of water entering the sponge by the pores or *inhalent* apertures, traversing the various channels in the substance of the sponge, and emerging from the oscula or *exhalent* apertures. Fig. 2, plate 10, shows, by arrows, the direction of these currents.

Sections of fresh sponge display the fact that the slimy substance—the so-called *sponge flesh*—consists of an assemblage of nucleated corpuscles or *sarcoids*, about $\frac{1}{200}$ or $\frac{1}{300}$ of an inch in diameter, and of irregular and inconstant form (see Plate 10, fig. 1., *e.*) Each consists of a speck of colourless protoplasm, the semi-fluid granular interior of which—the *endosarc* (Gr. *endon*, within; *sarx*, flesh) passes into a firmer clear outer layer—the *ectosarc* (Gr. *ektos*, outside). In the endosarc, besides the nucleus there is sometimes a little cavity—*contractile vesicle*—endowed with the power of rhythmic dilation and contraction. The sarcoid has the power of changing its form by the protrusion of blunt processes—from any part of its body, and when free from the mass it can crawl about by the same means. It bears, in fact, a remarkably close resemblance to an Amœba. In various parts of the canals, especially near the surface of the sponge, there are found round or oval chambers, lined with a layer of sarcoids, which present some advance in structure on the simple amœbiform type. Their form is usually columnar or oval (Plate 10, fig. 1., *b. c.*), they possess nuclei and contractile vesicles, and their outer layer frequently assumes the character of a distinct limiting membrane. Each possesses a long *flagellum* (Lat. for whip) which it is

capable of lashing backwards and forwards. In many forms the limiting membrane is raised up round the base of the flagellum into a membranous collar, and the sarcoid very closely resembles certain collared infusoria (Fig. 1.*c*.) Occasionally very peculiar forms are met with, as in fig. 1.*a*, where the flagellum and collar are borne at the end of a long neck. We can now understand that the currents which transverse the sponge are caused by the co-ordinated lashing in one direction of the flagella in these *ciliated chambers*, as they are called. But how is this co-ordination brought about? We do not know. It is one of the mysteries of protozoic life. Generally on the outside of the sponge, and less constantly in various parts of the interior, masses of nucleated protoplasm occur, which present a variation from the amoebal type in the opposite direction to that taken by the flagellated sarcoids, which we have seen is one of elaboration and specialisation. The masses in question present a degradation of structure, for the sarcoids of which they originally consisted have lost all their individuality, and fused into a continuous film, or *syncytium*, as Haeckel calls it, and all that remains to mark their presence is their nuclei, but the mass still retains its functional activity.

In some very few sponges (Myxospongiæ) there is no skeleton. In the others the skeleton is usually strengthened, and, in some cases, entirely formed, by spiculae of carbonate of lime or silica. These spiculae are of most varied and beautiful forms. In fig. 4 a few of the most characteristic of these are represented, but if the reader wishes to see what a great variety there is, he must be referred to Bowerbank's splendid monograph, published by the Ray Society, in which he will see two or three hundred distinct forms beautifully figured, and if he has nothing else to keep him out of mischief, he may occupy himself by learning by heart the two or three hundred names invented for their designation. Just to incite him to this study we quote three of these names—Exflected elongo-equiangulated triradiate, Furcated attenuato-patento-ternate, Torqueato-tridentate inequi-anchorate. We confess with all humility that we have not these terms at our fingers' ends. It is evident that these spiculae must tend to the preservation of the species possessing them, for by rendering them altogether unsuitable for the purpose of man, they are protected from his depredations, and they must enjoy an equal immunity from the attacks of other creatures who would otherwise prey upon them, for a mouthful of the spicules would by most animals be relished about as much as would a mouthful of their descriptive terms by the average mortal. In one order of sponges—the Calcispongiæ—the skeleton consists entirely of interlaced spiculae of calcic carbonate. In another order—the Fibrospongiæ, a fibrous keratode skeleton is always present, and in most forms siliceous spiculae are abundantly distributed (see fig. 3), not only through the keratode, but in the sponge flesh, Halichondria, a section of which is issued with this number, belongs to this order, and shows well the fasciculi of acicular spicules. The sponges of commerce are those forms of horny sponges which contain no spiculæ. In other sponges, such as the beautiful Euplectella, the spicules are very abundant and very closely united, and the quantity of

keratode is excessively small. Finally, in Clionia there is no trace of keratode, and the skeleton is entirely siliceous.

The reproduction of sponges is effected by a sexual process. Nucleated cells, exactly resembling the *ova* of higher animals are formed by certain of the sarcoids becoming detached, and acquiring a spherical form while retaining their nuclei and nucleoli, while certain other sarcoids undergo changes resulting in the breaking up of their contents into numerous minute bodies—*spermatozoa*, provided with long vibratile filaments, by which they can propel themselves through the water. Fertilization is brought about by the contact of one or more spermatozoa with the ovum, the interior of which then breaks up into two portions which sub-divide again and again, until the originally single cell comes to consist of a hollow oval chamber whose walls are composed of two layers of cells—an inner—*endoderm*, and an outer—*ectoderm*. At this stage the embryo is free, and swims about by means of cilia with which the ectoderm is covered. One end of the embryo then turns in and converts it into a hollow sac, and the *gastrula*, as it is now called, attaches itself by the closed end to some object at the sea bottom, and loses the cilia of its ectoderm, the cells of which unite closely with each other to form the syncytium. Pores appear here and there in the syncytium, through which inhalent currents are caused to set in by the cilia of the endoderm, and the water is discharged by the opening at the apex, which forms the single exhalent aperture or osculum. Before this period, however, spicules have made their appearance in the ectoderm, and young sponge has acquired a tolerably complete skeleton. Many of the Calcispongiæ remain permanently in this condition of a hollow chamber with thin walls and a single osculum. In the more complex sponges further development takes place mainly by the growth of the syncytium, whereby the endodermal cells become separated into small groups, which are ultimately restricted to the ciliated chambers.

In Spongilla, the only sponge inhabiting fresh water, an asexual process of reproduction also occurs. Certain of the amœbiform sarcoids retract their processes, become surrounded except at one point, by a spiculigerous wall, and after a period of rest are set free in the water and each reproduces the parent form.

The affinities of the sponges are very imperfectly understood, and they are among the most difficult animals to assign to their proper place in any system of classification The resemblance of the sarcoids to Amœbæ and Flagellate Infusors is so close, that from a study of the mature forms they would be unhesitatingly placed in the lowest sub-kingdom—the Protozoa. But among the Protozoa sexual reproduction is very rare, and a segmented ovum with the subsequent formation of a multicellular gastrula is a thing altogether unknown, while this character unites the sponges with the members of the next higher sub-kingdom—the Cœlenterata, from which, however, they differ very widely in their subsequent development.

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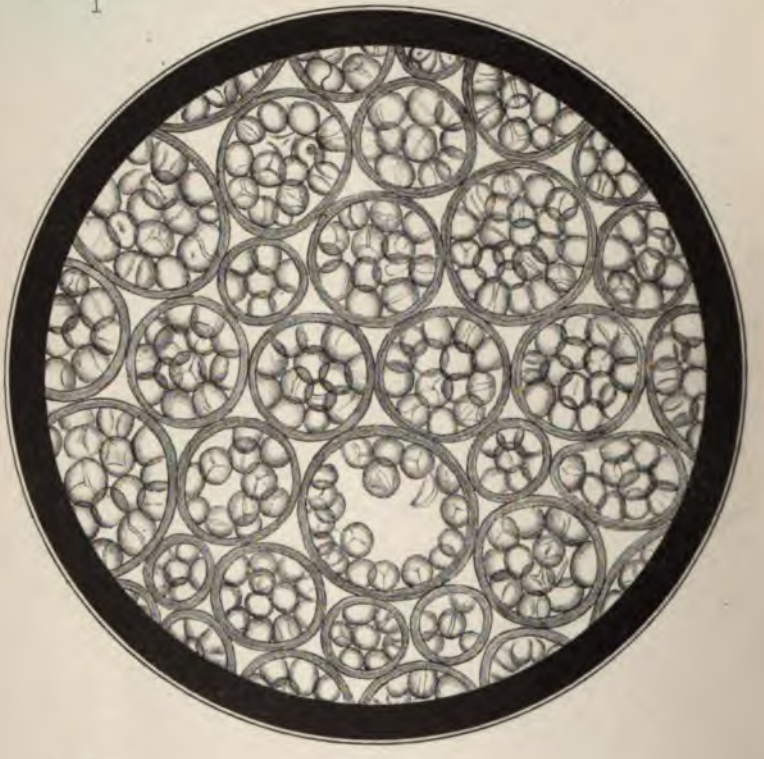

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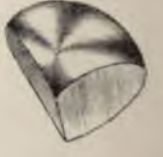

6

P. C. del. ad nat. J. W. Watson. del. et lith.

STARCH

Sarsaparilla officinalis.

X 400

Watson & Son, Lith. 313 Charles St. Boro.

STARCH.

Description of Plate.

Trans. sect. root of *sarsaparilla (smilax) officinalis*, showing medullary parenchyma with intercellular passages, and starch grains in situ in cells. Ordinary light, × 400·1-6. Detached grains and granules as seen under the same magnifying power, but drawn on a larger scale. Crossed Nicols. 1. Simple grain, showing striation, one zone being more distinct than the rest. 2. Compound grain of irregular form, showing two of the component granules, Side view. 3. Compound grain. End view, showing the three granules. 5. Side view of compound grain of regular form. 4 and 6. Detached granules.

When exposed to sunshine, all green parts of healthy living plants, and especially the leaves, are the seat of remarkable chemical activity. The air, with its small but all-sufficient quantity of carbon dioxide, which has gained access to the green cells by means of the stomata and the intercellular passages, and the water forming the chief constituent of the cell sap, being brought into close relation to each other there result a series of most important synthetical operations which cannot be brought about by any other means known to chemists Under the influence of the sunshine and the chlorophyll the carbon dioxide ($C O_2$) is decomposed, its oxygen is liberated, and the carbon and the water unite with each other directly, in certain proportions, to form organic compounds of greater or less complexity, which are used by the plant for the growth of the young cells, and the nutrition of all living parts. Among these first products of *assimilation*,[1] as this process is called, are sugar, oil and fat, and *starch*. When assimilation is in excess of the present demands of the plant, the excess of nutrient matter is removed from the cells in which it is formed, and stored up in other parts for future use. When the first-

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[1] This term is here used in the perverted sense customary among botanists. Properly assimilation is the process by which the prepared nutriment is incorporated with the actual substance of the plant, and becomes part of its body.

formed products are soluble in the cell sap, their transmission is of course readily explained by the constant circulation of sap, but when insoluble the transmission must be otherwise accounted for. The substance, in fact, must undergo a process of *metastasis*, or conversion into some soluble body, and this, on its arrival at the place of deposition, may be reconverted into its original insoluble state, and so remain until it is required for use.

Of these substances *starch* is by far the most important. It is one of a class of bodies called carbo-hydrates, that is, it consists of a compound of carbon with oxygen and hydrogen in the proportion of 1 part of oxygen to 2 of hydrogen, or in other words, in the proportion in which they exist in water. So we may regard starch as a compound of carbon and water. This fact should be borne in mind in connection with its use as a dietetic, for its hydrogen being already completely oxidised or burnt cannot contribute to the production of heat in the body, and so does not take equal rank as a heat producer with the fats and oils in which the hydrogen as well as the carbon is in excess. Starch is insoluble in water and cell sap. When analysed it yields the proportional constitution $C_6H_{10}O_5$, but these figures do not represent the true complexity of its molecule, which is best represented by $nC_6H_{10}O_5$, in which n is used in the algebraical sense to stand for an unknown quantity, but probably $=3$, so that the true formula would be $C_{18}H_{30}O_{15}$. It is closely related to *glycogen* or liver starch, *dextrin* and *cellulose*, and although all these bodies differ in their physical and many of their chemical properties, they have exactly the same composition, or, as chemists say—they are *isomerous*. From glucose or grape sugar, the formula of which is $nC_6H_{12}O_6$. It differs only in the possession of one molecule less of water, and is easily convertible into that substance. Treated with a very dilute solution of *iodine* starch assumes a purplish blue colour, and this reaction being very delicate and highly characteristic of starch, affords the most valuable test for that substance, even the optical one to be presently mentioned not excepted. It has been usually assumed that the iodine formed a definite compound with the starch (the so-called iodide of starch), but there is reason to believe that it is only deposited on the starch in a metallic state.

By the action of an organic nitrogenous body of very doubtful composition, called *diastase*, produced in germinating seeds, and by other means, starch is converted into dextrin and glucose, both of which are readily soluble in water or the cell sap. This, of course, is important in explaining how the insoluble starch may find its way from the chlorophyll grains, in which it originates, to the cells where it is stored.

Starch is first formed in the interior of the chlorophyll grains as minute, rounded, solid particles. During the whole time the green leaves are exposed to sun light, an accumulation of these particles occurs in the chlorophyll grains, but as soon as the light fades the quantity

which has been accumulating all day decreases, and the starch is gradually removed in the form of dextrin, or some other soluble form, then on reaching the receptacle in which it is to be stored an inverse change ensues and the dextrin is once more converted into starch, in which form it is deposited. Although the process of removal of the original particles of starch from the chlorophyll grains can be traced only at night, it, in all probability, goes on constantly, but during the daytime the rate of removal is insufficient to counterbalance the larger amount of new material formed.

In the chlorophyll grains the particles of starch never reach any higher degree of organisation than that mentioned above, but in the stems, tubers, roots, rhizomes, seeds, and other parts where it is stored for future use, it assumes the form of complex and definitely-organised grains, whose form is characteristic of the genus or species in which they occur. The grains are frequently of a large size, but the size varies considerably, being in some plants almost immeasureably minute, in others as the Potato and Tous-les-mois, attaining a diameter of as much as a four-hundredth of an inch, and being readily visible with a simple lens. In the same plant, and even the same cell, the size varies considerably, being dependent chiefly on the relative age of the grains, so that when measures of starch grains from various sources are given, they must be taken only to represent an average, and much latitude must be allowed for individual variation. In some plants, though—the Sarsaparilla for instance—the variation in size is less marked. The forms of the starch grains are as variable as their sizes. In the potato they are oval, in the bean elliptical, in some orchids spherical, in the wheat grain lenticular, in the maize polyangular, in ginger root, like short bent rods, and in the laticiferous cells of Euphorbia peculiar bone-like forms occur. In the oat the grains are compound, consisting of a number of closely packed, but readily separable, granules.

Examined under a sufficiently high power, and in a suitable medium (50 per cent. glycerine answers well), a dark spot will be seen in most grains. This is called the hilum or nucleus and is usually placed eccentrically. In those grains which are elliptical, it is placed nearest the narrow end of the grain. Surrounding the hilum will be observed a number of zones, alternately light and dark, due to alternations of more and less watery layers, and besides these alternations of much and little water in the layers, there is a progressive increase in the quantity of water or decrease in the density of the layers from without inwards, the hilum being always the darkest and most watery part. That this is a correct explanation of the appearance of the layers may be seen by observing the grains in a medium—alcohol, for instance—which abstracts the water, and entirely, or almost entirely, obliterates the appearance of zoning. When the grains are allowed to dry, the striation is also very indistinct, and the place of the hilum is then usually seen to be occupied by a cavity containing air. A stratum of air also sometimes occurs be-

tween two layers, and brings them into view when they would otherwise be invisible. In the dry grains, too, a number of cracks may be seen radiating from the hilum, and produced by the greater shrinkage of the central parts of the grain in consequence of the greater loss of water from them than from the outer layers.

Concerning the mode of growth of the grain, and the origin of the layers, there has been, and is, much dispute. One set of observers state that the growth takes place as in a crystal, by accretion or deposition of layers, alternately more or less hydrated, on the outside of the grain, so that the outermost layers are the youngest. But Sachs, following Naegeli, maintains that the grains grow by intussusception or deposition of molecules in the interior of the grain between already existing molecules, and weighty arguments are adduced in favour of this view.

The excentric position of the hilum is thus explained. The starch is never deposited in entirely dead and empty cells, but usually in cells whose vital activity is reduced, and but a lining zone of protoplasm remains. It is in this layer that the growth of the grain commences, and as it increases in size it is pushed towards the centre of the cell and is no longer entirely surrounded by protoplasm, and the part so removed from contact with the protoplasm naturally grows more slowly than the side that is still imbedded. Sometimes the starch is deposited in hollow vescicles in the protoplasm, then as growth takes place the grain extends into the cavity away from the protoplasm, and the same irregular growth as before results.

The hilum commonly conforms to the shape of the grain. In elliptical grains it is elongated in direction of the longest axis of the ellipse, and in lenticular grains the hilum is lenticular. Occasionally, two or more nuclei appear in one grain, and concentric layers are deposited around each. This is often seen in the haricot bean. When it occurs the most rapid growth usually takes place in the line joining the two nuclei, and a rupture at length takes place, whereby the original single grain becomes divided into two, though they may still remain in contact with each other. Such compound grains occur in Sarsaparilla. Examined under a quarter or eighth inch object glass. The cells of the cortex and medulla will be seen to be filled with rounded grains, most of which show traces of a division into three separate granules. In this balsam preparation it is not easy to see the striation for the reason above given, but some grains are sure to shew it under a suitable illumination. It will then be seen that the layers do not encircle the grain as a whole, but each granule has its own hilum surrounded by its own concentric layers. Very often one or two divisions are much more pronounced than the rest, as shewn in fig. 1 plate 11.

Starch assumes a most characteristic appearance under polarised light. Space will not allow, nor is it needful to enter into an account of the

nature of polarised light, with which we must assume our readers to be in some degree familiar. It will suffice here to draw attention to the phenomena presented by its use. When starch is examined under crossed nicols the field remains dark, but each granule assumes a glistening grey appearance, as if self-luminous, and is marked with a black cross. If, then, the object be slowly rotated in the field of view, it will be seen that the cross remains fixed with regard to the field, one pair of its arms being parallel to the principal plane of the polariser and the other parallel to the principal plane of the analyser. As the arms of the cross, however, are frequently curved, their direction does not always appear to coincide with these planes. During the rotation, in fact, the grain appears to be turned round underneath the stationary cross.

If, the object remaining stationary, the polariser or the analyser be rotated, the cross will be seen to rotate with it but with only half its angular velocity, so that to make a complete rotation of the cross the analyser or polariser must be rotated twice. If now a thin film of selenite be interposed between the polariser and the object while the nicols are crossed, and be rotated until it gives the brightest field, most beautiful chromatic effects will be obtained. The field will assume a colour dependent upon the thickness of the selenite film, and the interference crosses will be vividly coloured, the rest of the grains assuming a complementary colour. For instance if a yellow-blue selenite be employed and be so adjusted in the first instance as to give a blue field the crosses will be red at the edges merging into yellow in the centre, and the interspaces will be bright green. Then on rotating the analyser or polariser, as the blue field gradually merges into the complementary yellow, so the crosses rotate and change to their complementary colours. By means of the interference phenomena under polarised light, the compound nature of the grains in sarsaparilla is most clearly shewn, for each granule exhibits its own cross and its own chromatic effects. In studying this preparation, the medulla or pith is the most favourable part, for here the cells are largest and the starch less closely packed. Some grains will be met that are not compound and exhibit but a single cross, as shown in fig. 1. Others will be presented under different aspects, some showing the triradiate division of the granules as seen in plan (see fig. 3). Others a single diametral suture when the grain is seen from the side as in fig. 5. Frequently the normal spherical form is departed from (fig. 2). Round the edges of the cells especially, the grains have, by mutual pressure, assumed the form of very short truncated cones with rounded angles. This is represented in the central figure. If the observer searches the cells carefully he will probably be rewarded by finding a few isolated granules, the appearance of which will of course vary according to their presentment, and these will afford a better idea of the true form of the granules than could possibly be obtained by any other means. Occasionally grains are met with having more than three component granules.

The South American genus Smilax, from the roots of several species of which the sarsaparilla of the Pharmacopeia is obtained, belongs to the natural order Smilaceæ, which together with a few other orders, presents a remarkable departure from the normal type of monocotyledons. The form of the embryo and of the flower, the minute structure of the ærial stem and its branches, and the general character of the plant are exactly those of other monocotyledons, but the veins in the leaves form a network and the *rhizome* or creeping underground stem has its woody tissue disposed in a ring round a central pith or *medulla*, and is surrounded in turn by a parenchymatous cortical layer. These characters are as distinctly dicotyledonous as those before mentioned are monocotyledonous.[1] The arrangement of the bast and xylem in the root is somewhat different, for instead of each bundle consisting of an internal woody portion separated by a cambium ring from an external bast portion the two constituents are arranged collaterally, large xylem bundles consisting of large vessels and thick-walled prosenchymatous cells alternating with much smaller bast bundles composed in the main of sieve tubes, the whole being surrounded by a single layer of very thick walled cells, representing the vascular bundle sheath. Under a 1 inch objective this arrangement will be well seen in the accompanying preparation, and a $\frac{1}{4}$ inch applied to the sieve tubes will show here and there the perforated end walls or sieve plates between the ends of adjacent vessels. All the elements of the root are highly lignified, and polarise strongly without the aid of a selenite.

[1]Contrast this with the trans. sect. of the Bulrush, which is an ordinary monocotyledon.

E.T.D. del. ad nat.

T. S. OF DODDER (CUSCUTA)
in its host, double stained.
X 75

Watson & Son, Tech. Rd. 6 Charles St. Birm.m

THE DODDER PLANT.

The Dodder plant is a long and slender-stemmed leafless parasite, rather plentifully distributed throughout most of the counties of England, but totally unknown as a native in either Scotland or Ireland. It selects as a host either a shrubby plant, as in the case of the individual from which the accompanying section was prepared, or, as is more common, it fixes itself upon certain wild or cultivated plants, such as thyme, nettles, clover, or flax. It blooms in the summer, the flowers being in the form of small white or pinkish bells, growing in close unstalked globular clusters, and passing later into roundish capsules, each containing four small, brown, and minutely granular seeds. The plant attaches itself by twining its pale or reddish thread-like stem around the body of its selected prey, and by sending out, at intervals, along the encroaching filament, peculiar adventitious roots, suckers, or haustoria, which find their way into the sap-containing tissues of the host-plant, and not only function as holdfasts, but, at the same time, also act as ready absorbents of the nutritive juices, that, during the growing season, are ever circulating within the body of a living plant.

The Dodder, which supplies the subject of our present study, has selected as its host a plant of common heath, but several other plants—in addition to the few mentioned above—are well known as affording it a suitable habitat. Furze, broom, cranberry, and rockrose are the best known examples of shrubs; bastard toadflax, camomile, sowthistle, yellow rattle, and bracken fern as wild herbaceous plants; while cabbages, hops, and lucerne may be added as further examples of the cultivated plants that are subject to the attacks of this agricultural pest.

Dodder belongs to the genus *cuscuta* of botanists, a word said to be derived from *chessuth* or *chasuth*, the Arabic name for this plant. There are about 80 different kinds or species of cuscuta distributed throughout the warm and temperate regions of the earth, and of these four may be found in England. The largest and rarest grows upon vetches, nettles, tansy, &c., and is distributed generally throughout temperate and sub-tropical Europe, and is known as *Europæa*. Another form as large possibly as *Europæa*, but not so red, grows upon flax. It is not a true native, having been introduced in imported linseed, and may be found in flax fields in any part of the British Isles. It is named *Epilinum*, from *Epi*, signifying that it grows upon, and *linum*, the Latin name for the flax plant. The third form or species of Cuscuta affects thyme; it possesses a very slender stem, and has a European distribution from Denmark southward, extending into North Africa and Western Asia. It has been named *Epithymum* from Epi and *thymum*, the Latin name for the herb thyme. The species of our preparation is *Cuscuta Epithymum*, and it not only grows upon thyme and heath, but upon ling, furze, and other plants, woody and herbaceous. The fourth form of Dodder found in England may be regarded as a mere variety of the last. It occurs in clover fields, and is a most troublesome weed to the agriculturist. It is known as *Cuscuta trifolii*; trifolium (three-leafed) being the generic name for clover.

Cuscuta is known in different parts of the country, by names considerably more expressive of the parasite's look and behaviour, than the

polite and gentle one of "dodder," which simply means a tangled thread. Strangle-weed, hell-weed, and Devil's-guts are common names given to it in agricultural districts.

From the structure of the flowers, or reproductive organs of the plant, a natural relationship with ordinary field or garden convolvulus (bind-weed) can be clearly traced; as in these structures, which were developed at a comparatively late period in the life of the individual, and structures, too, that are in no way related (other than a relation of dependence) to the purely vegetative functions of the plant, they have not suffered that degeneration of structure that has befallen the nutritive organs, in consequence of the plant having taken to the ever-degrading habit of parasitism. As the plant became more and more better fitted to lead a parasitical life, organs that were absolutely essential for the performance of the every-day physiological work of ordinary plant life became useless, then rudimentary and at last completely lost; thus the leaves not having occasion to elaborate food are reduced to the merest rudiments—tiny scales upon the blanched or red-stained stem; while the true roots have altogether disappeared—the plant at no time in its existence having been rooted to the earth. The flowers have five united petals and five stamens inserted, as in convolvulus, at the base of the corolla bell and alternate with its lobes. The ovary is superior, or placed in the receptacle above the insertion of the corolla, and consists of two cells or cavities each containing two ovules—further points of floral structure that clearly indicate an affinity with the bindweed. The genera *Convolvulus* and *Cuscuta* are the only British representatives of the family CONVOLVULACEÆ of botanists.

When the seeds of Dodder are fully ripe they each contain a slender embryo coiled around a central mass of reserve food, or endosperm. The two cotyledons, so characteristic of the class (DICOTYLEDONS) to which the family Convolvulaceæ belongs, are, however, not distinguishable in this plant. The embryo consists simply of a thread, stouter at one end than the other, the thicker extremity representing the primitive root or radicle. When, in the course of time, the seed germinates, the slender or plumule end of the embryo bursts the testa and rapidly lengthens, fed by the nourishment stored away in the seed, and absorbed by the fleshy radicle. The tip of the sensitive plumule moves round and round, performing circular journeys in search of a suitable host; having found one, say a young clover, flax, or other plant, it twines around it, and is eventually carried upwards, seed and all, completely off the ground, by the rapidly elongating host. By the time the store of endosperm matter is exhausted, the infant parasite has pierced the thin skinned body of its prey, and henceforth the suckers provide it with already elaborated food, equal in value to that which was stored away within the old, but now cast away seed-coats by the parent dodder plant.

How these suckers act will be best explained and easier understood after a careful examination is made of the histological relationships existing between the tissues of the host and its parasite, as exposed in the accompanying preparation. It will be well to examine first the structure of the stem of Heath.

In the centre, as displayed by the transverse section, is the region of pith, the cells of which are relatively large and many-sided. Examined in a fresh state and in sections not too thinly cut, the walls may be seen to be closely pitted with small roundish pits or depressions left in the slightly thickened side walls. These cells are utilised by the heath plant for the storage of food, and starch grains may be often observed lying within them. Surrounding the pith is the region of wood, or as it is sometimes called the xylem, made up of narrow thickened fibres closely intermixed with long wide tubes or wood vessels. It is up this fibro-vascular cylinder that the water, charged with the essential earth salts, passes conducted especially along the woody fibres, while the long pipes or vessels serve as channels for the interchange of gases, rendered necessary by the chemical changes induced by vital energy at the growing or working regions of the plant. Lying around the woody fibro-vascular cylinder is the cortical layer or region of bast, a tissue also composed of fibres and vessels. But the elements of the bast fibro-vascular cylinder differ not only in structure from those of the wood, but in certain chemical and physical particulars as well. The function, moreover, of the two regions is essentially different. The vessels of the bast, instead of being mere passive conductors of gases, are tubes containing living protoplasm, and are active distributors of sap relatively rich in albuminoids.

Outside of all is the protecting layer of corky cells, covered externally with an epidermis, from which spring numbers of comparatively long, roughish-looking hairs.

Respecting the structure of the Dodder, it is extremely simple. As displayed in longitudinal section, it will be observed to consist of a vascular system, the elements of which are barred with transverse thickenings, and the whole surrounded by a large-celled parenchymatous tissue, abundantly filled with grains of starch. There does not appear to be a very distinct epidermis. The suckers are apparently of the same general structure as the stem, and, in the preparation, are seen in position as being thrust through the superficial protecting layer of the host, and embedded either in the tissue of the bast, or pushed inward through the wood as far even, in some cases, as the pith.

Remembering that the function of chlorophyll in the vegetable economy is in connection with the manufacture of starch, it is not strange that in the Dodder this important green substance should be entirely absent, seeing that it can collect starch or its chemical and physiological equivalent from the passing juices of its host. In all likelihood the protoplasm contained in the superficial cells of the haustoria exercise an influence similar to that of the scutellum in the germinating wheat, enabling the insinuating suckers to not only absorb already soluble materials or substances dissolved in the sap, but solid material like grains of starch and protein bodies in addition. Furthermore, it is by this power, one might conjecture, that the haustoria can gain an entrance, and push their way into the stem of the host. When it is remembered how the embryo of the date for example, can soften and absorb during the period of germination the

exceedingly hard and dense endosperm substance of its seed, one cannot wonder at the power possessed by the suckers of the Dodder to enter by the exercise of a similiar digestive power, even the hard woody cylinder of shrubby plants like Heath. At all events, it is hardly likely that the suckers, even although they *are* provided with a twig from the fibro-vascular system, "pierce the stem [of the host] like a small thorn," or again that the "suckers could not penetrate the clover stem were it not for the woody skeleton belonging to each sucker," as is described in a recently published text book.

Parasitism although common among Fungi is comparatively rare in the rank and file of Flowering Plants. In the class of Dicotyledons there are no parasites among the forms with free petals, but in the *Monopetalæ*, or those whose flowers have united petals, and in the *Apetalæ*, or those where there are no petals at all, there are several families possessing parasitical individuals. Confining ourselves, however, to members of the British flora, we have the following list:—

MONOPETALÆ.—Dodder *(Cuscuta)* of the order Convolvulaceæ Bird's-nest *(Hypopithys* or *Monotropa)* of the order *Monotropeæ* found in woods upon roots of beech and fir.

Bartsia, Eyebright *(Euphrasia)*, Yellow-rattle *(Rhinanthus)*, Lousewort *(Pedicularis)*, Cowwheat *(Melampyrum)*, and Toothwort *(Lathræa)*, all of the order Scrophulariaceæ, and all parasitical upon roots.

Broom-rape *(Orobanche)* of the order Orobancheæ, parasitic upon roots.

APETALÆ.—Bastard Toad-Flax *(Thesium)* of the order *Santalaceæ*, a perennial and parasitic upon roots.

Mistletoe *(Viscum)* of the order LORANTHACEÆ, parasitic on various trees.

In the class of MONOCOTYLEDONS we have Coral-root *(Corallorhiza)* and Birds'-nest Orchis *(Neottia)* root-parasites of the order Orchidaceæ. *Epipogum* of the same order is a saprophyte, that is, it feeds upon organic matter derived from lifeless things. It lives amongst decayed leaves and is exceedingly rare.

In this list two or three plants are given that can only be regarded as partial parasites. Mistletoe, for example, although it feeds upon the juices of apple and other trees, its leaves nevertheless contain chlorophyll, and, within certain limits, manufacture starch from carbon di-oxide and water just as other green plants do. Its usual mode of procedure therefore is to add to the nutritive store enriched by legitimate labour, that surreptitiously obtained by sucking the food-bearing sap of its woody host. Rhinanthus and Thesium differing from Mistletoe in being only parasitical upon roots, the former affecting damp and the latter dry and chalky pastures, and the other British examples of parasites that still retain the power of developing chlorophyll in their leaf cells.

The most remarkable parasite, and, indeed, the most remarkable plant perhaps in the whole world, is the *Rafflesia Arnoldi* of Sumatra, an individual consisting entirely of a huge flower of fungoid consistency, provided with a single sucker which attaches the plant to roots of certain species of *Cissus*, trees belonging to the same natural family as the vine. The flower of this leafless and stemless parasite actually measures three feet in diameter, and weighs upwards of fourteen pounds.

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SECTION III.

POPULAR MICROSCOPICAL STUDIES.

PAGE 24.—ll. 9 and 15, from top; for "albuminoids" read "albumenoids."

PAGE 27.—l. 7 from foot; for "stomatæ" read "stomata."

PAGE 42.—l. 5 from foot; for "madibles" read "mandibles."

PAGE 45.—l. 21 from top; for "spiculæ" read "spicula," and the same error is repeated several times in this page.

PAGE 46.—l. 23 from foot; for "and young sponge, &c.," read "and the young sponge, &c."

PAGE 50.—l. 10 from foot; "dele" full stop after object-glass, and capital T from next word. The sentence should read "Examined under a quarter or eighth-inch object-glass the cells of the cortex, &c."

THE METHODS OF MICROSCOPICAL RESEARCH,

AN INTRODUCTORY ESSAY

TO STUDIES IN

MICROSCOPICAL SCIENCE,

EDITED BY

ARTHUR C. COLE, F.R.M.S.

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THE METHODS

OF

MICROSCOPICAL RESEARCH.

Introduction.

I.—On Instruments and their Uses.

The investigation of minute structure, whether organic or inorganic can only be accomplished through the use of adequate optical instruments. The organ of vision in man is so constructed that the distance of most distinct vision wavers between eight and ten inches from the normal eye of an adult. This is due to the alterable curvature of the crystalline lens which permits of the practically parallel pencil of rays from a distant object or the divergent rays, from one close to the eye, to be accurately focussed on the retina or sensitive part of that organ; but if the object be brought still closer to the eye than the normal distance of most distinct vision, it gradually loses its power of accommodation in exerting the necessary strain which is required to render the crystalline lens convex enough to focalise the image of the object upon the retina; about two inches from the eye this strain becomes impossible. Within the limits of accommodation only megascopical characters can be appreciated. Were the eye capable of altering the curvature of its crystalline lens indefinitely, its power of vision would become illimitable; telescopes and microscopes would then be found unnecessary But the bounds of natural limitation can be conquered by artificial means, and the interposition of a sufficiently convex lens between the near object and the eye, alters the direction of the rays of light which proceed from the object, so as to bring them within the scope of natural vision; and herein lies the theory on which the microscope has been constructed. The nearer an object is brought to the eye, the greater does its visual angle (or angle produced by the intersection of rays or straight lines from the extreme points of the object) become, and, consequently, a larger image is focussed on the retina.

Optical instruments then are required at the outset to enable one to enter the domains of histology; they may be directly employed, in a multitude of instances, where the objects are of minute size. Equally vast in number, however, are the substances which cannot be directly examined, but require special processes to render them suitable for mi-

croscopical examination. In the inorganic kingdom minerals, rocks and chemical substances, elementary or otherwise, are found in nature in such minute particles as to render them suitable for immediate study under the microscope; but, in the majority of instances, they require to be pulverised, sliced, or precipitated ere they come within the range of observation, and to these ends the petrologist must be provided with chisels and hammers, slicing and grinding apparatus, and a variety of other tools adapted to the collection and subsequent treatment of specimens, whilst the chemist must stock himself with blowpipes, test tubes and matrasses, reagents, and balances. In the organic world the vegetable histologist will require a spud and vasculum, dipping bottles, collecting apparatus of all kinds, and a variety of instruments to facilitate the examination of the unicellular and delicate forms, whilst the more complex examples will require to be dissected before they can be utilised. The animal histologist will find his work inextricably linked with that of his brother botanist, and to a large extent will work hand in hand with him; but in the progression of his inquiries there will arise a necessity for other instruments, such as bone-forceps, saws, and knives of divers shapes.

The study of both organic and inorganic histology is so complicated, that words alone are often inadequate to its wants. Diagrams and drawings are therefore employed to supplement what language often fails to express. There has thus arisen the necessity for instrumental aid in this direction, and it becomes the duty of the investigator to learn their construction and use.

II.—On Reagents, their constitution and action.

One of the most important items in the study of microscopical technology, is concerned with the action of reagents. It often happens that the optical means at the disposal of the histologist are insufficient to the resolution of structure, and although much may be done with carefully-directed and modified light, still, the utility of the judicious application of reagents requires only to be understood to be appreciated.

The knowledge of the chemical constitution, physical properties, and modes of manufacture of reagents, often leads to the discovery of their specific action on minute structure, whether those consist in a revelation of inherent qualities, or in a modification of details. It thus becomes incumbent that the histologist should carefully scrutinise all kinds of reagents, and record the results of such examination.

Reagents may be used in a variety of ways, for the elucidation of structure and the detection of the constitution of bodies. Some are employed as chemical tests, others as stains or to induce changes whereby certain properties are revealed, whilst another set are exhibited as preservative media. To know exactly what to apply, and how to apply it for the revelation of specific phenomena, is the essence of this department of microscopical manipulation.

III.—On the Methods of Preparation.

Both organic and inorganic matters require special methods for their preparation as a means of study. Thus, the processes of pulverisation, levigation, of slitting, and of grinding minerals and rocks, are beset with difficulties of detail, which, for want of suitable attention, prove to be insurmountable barriers to the tyro, whereas their observance but shows that he has made a "mountain of a mole-hill." So, also, the impediments to successful section-cutting, staining, and mounting are all traceable to a neglect of minute particulars, such as the wetting of the edge of the razor with spirit, the practice of drying the edge of the blade when it is set down for a few minutes, the use of a mordant previous to staining certain vegetable tissues, or the thorough dehydration of sections before they are mounted in Canada balsam or dammar solution.

In the opening pages of his work on the microscope,[1] Beale makes the following observations:—"Manual dexterity, although subordinate to many higher mental qualifications, is as essential for the successful prosecution of microscopic observation as it is for that of every kind of experimental science. It assists us in the discovery of new means of enquiry and in devising methods by which difficulties may be surmounted. Without skilful manipulation we can neither teach by demonstration facts which have been already discovered, nor hope to extend the limits of observation and experimental knowledge. It is not, therefore, surprising that many of the most important facts which have been recently added to microscopical science, have been discovered by men who had previously well-trained themselves in experiment—particularly in practical chemistry and minute anatomical dissection. Improvements in the practical details of manipulation almost necessarily precede an advance in natural knowledge, and invariably promote and expedite true scientific progress."

But although manipulative skill is a very necessary adjunct to microscopical research, an attainment of the understanding of the general principles of action at the outset, sometimes proves to be the most arduous portion of the work, and very often is the only impediment to success. Practice and perseverance, brought to bear upon previously gained knowledge, are the only royal roads to manual dexterity, and it thus becomes the duty of the instructor to point out, not merely what path ought to be taken, but the various pit-falls which everywhere surround the beaten track, and how best to avoid them.

IV.—On Microscopical Art.

There are two ways in which microscopical objects can be drawn so as to become useful records of research. By the first of these, a rough diagrammatic representation may be made, without reference to accuracy of

[1] *How to Work with the Microscope*, 5th Ed., London, 1880, p. 1.

form or size; merely to display the author's views concerning the structure of the object. The second method is to make an accurate drawing with due regard to the size and shape of the object under the microscope. Both of these methods are valuable in themselves, but their usefulness becomes immeasurably enhanced when they are combined so as to afford scope to the artistic skill and scientific knowledge of the draughtsman.

There is a great deal of truth in the statement that true art is the outcome of genius, but that does not in any way affect microscopical drawing; it will be found that patience and practice are sufficient to enable the student to overcome every obstacle, and to achieve the most satisfactory results in this department of art. Photography, and lithographic drawing, also, need only to be attempted, to convince the worker that perseverance, here as elsewhere, is the only serious impediment to success.

In the ensuing chapters every general statement will be followed by some special example, and the study of types thus afforded, will endow the beginner with material for extended microscopical research.

On Instruments and their Uses.

CHAPTER I.

THE MICROSCOPE.

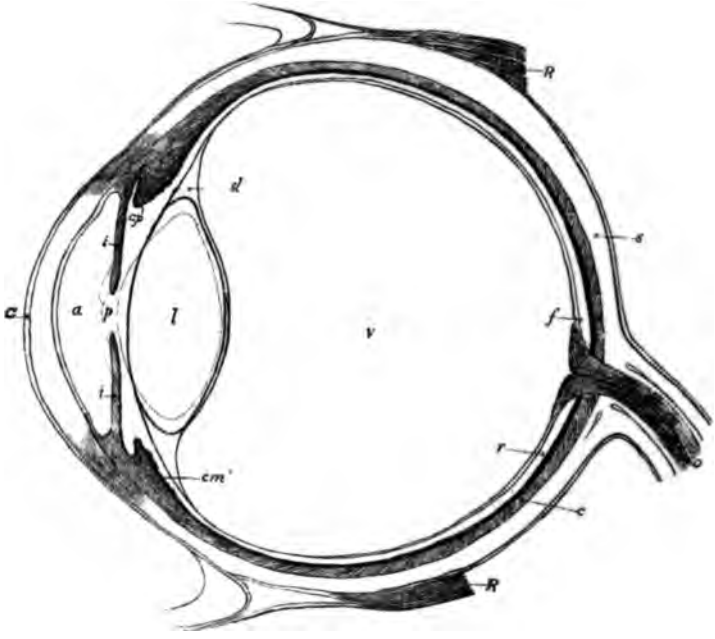
The microscope, as an instrument of power in histological research, depends essentially in construction, upon its conformity with the laws of light and human vision. It has already been stated that an idea of the size of an object is arrived at through the size of the image focussed upon the retina, and that these dimensions vary in proportion as the object is brought near to, or removed farther from, the eye. When the object is brought close to the eye, its visual angle,—that is the angle formed by the crossing of the rays from the extreme points of the object, is larger than when it is placed farther off, and, consequently, the image on the retina is larger also. If this principle were capable of unlimited extension, it would obviously follow, that to keep on magnifying an object, all one would have to do would be to bring the object closer and closer to the eye. But there is a limit to this natural power of microscopical vision in the human subject, and the eye fails signally to accomplish its office when the object is brought within about two inches from its surface. The reason of this is, that, the crystalline lens of the eye, in assuming a more convex shape through the relaxation of the ciliary muscle, becomes overtaxed at this distance. If, now, a sufficiently convex lens be placed between the object and the eye, so as to enable the divergent rays to be accurately focussed upon the retina, the difficulty will be overcome, and, theoretically, microscopical vision would be illimitable. But, is it so? Most certainly not The employment of artificial substances, such as crown and flint glass, diamonds, etc., although they considerably extend the power of sight, do not do so *ad infinitum*. Here the limitation is purely material, as distinguished from the former instance, the human eye, which is defective not only materially, but physiologically.

The worker in the field of microscopical research, need not, however, be appalled by these statements; for, it will be found that the human organ of vision, in conjunction with the excellent appliances of modern invention, will enable him to approach, and sometimes even to solve satisfactorily, many of those philosophical problems which underlie the evolution of things, both animate and inanimate. In exemplification of this, a few instances may here be recorded.

The practical geologist who sallies forth into the field with lens in hand, may gather during his walk a variety of rocks, which, from their cosmological structure, point to an igneous origin; some of the specimens are coarse grained, whilst others defy the utmost scrutiny of the eye. The microscope is brought to bear upon the question, and he finds that a power of 500 diameters is generally the utmost degree of amplification he will require to employ; but for all practical purposes powers of from 20 to 100 diameters suffice. With the assistance of the microscope he is enabled to pronounce with decision that the rocks are igneous; and more, from analytical and synthetical experiments he can show that certain coarse varieties, which are thoroughly crystalline (the crystals being simply held together by adhesive and cohesive forces, without the necessity of an interstitial binding substance), are of deep seated origin, and consolidated under conditions of enormous pressure and a length of time. He is, in like manner, able to affirm of the other varieties, what their mineral constituents are, or have been, and how they came to assume their present states. Thus he builds a part of the fabric of geological philosophy, and with what?—with a comparatively low power of the microscope.

To take an example from the organic world; the questions of the function of various parts of the body, are very often arrived at through a minute study of its members. The form and general appearance of the cells of glands such as the salivary glands, point to the functions they perform, whether they secrete or absorb, and how and when they perform their duties. The study of amœboid, and even of ciliary motion, under the microscope, does not require a power of magnification, much beyond 700 diameters, whilst the life-history of the minute forms of life known as germs (*Bacteria, etc.*), may be readily comprehended, by the use of from 700-1200 diameters. It is only when such things as the delicate markings on the skeletons of Diatoms, or the artificial ruled lines on glass (Nobert's test) require to be made out, that powers beyond 1,200 diameters become useful; and it must be admitted that the scientific investigator does not lose anything, nor are any of his philosophical deductions vitiated by eschewing such powerful instruments, which, in the hands of the skilful, succeed in amplifying and resolving certain pretty structures; but, to the ordinary worker are, in reality, impediments to research.

On the threshold of this inquiry, it thus becomes evident, that an acquaintance with the general structure of the human eye, coupled with the principles of luminous energy, are necessary adjuncts to an understanding of the microscope its place, and power.



J. del. ad. nat.

Watson & Son, Chromo-Lithogrs. Northall St. Bath.

THE HUMAN EYE.

Slightly altered (by permission) from a paper read before the Manchester Microscopical Society, by GEORGE E. DAVIS, F.R.M.S., F.I.C., F.C.S., &c.

DESCRIPTION OF PLATE I.

a, Anterior chamber of eye fitted with the aqueous humour. *c*, Cornea. *i*, iris. *p*, pupil.
cm, Ciliary muscle. *cp*, ciliary processes. *l*, lens.
s, Sclerotic Coat. *c*, Choroid. *r*, retina.
f, Forea. *RR*, Superior and inferior recti muscles.
O, Optic nerve entering the sclerotic and choroid coats.

In all studies, whether of pure Microscopy as a Science, or whether of one of those departments of Natural History in which the microscope is employed as an aid to vision, we must, at the outset, recognise the importance of a study of the human eye.

It may be the seat of many imperfections resulting from misuse, old age, or disease, which are apt to modify the conclusions we may draw from our observations, unless we are careful to study well into what lines such imperfections may lead us.

Nature has given us in this organ a means whereby all objects may be compared with each other, more especially as to size, colour, and general characters, and it must astonish the student, who thinks deeply, to find that so little is known definitely as to *how* we are able to appreciate magnitudes, colours, and forms. It is easy to say that the eye lenses focus a picture of the object upon the retina, and the irritations are carried by the optic nerve to the brain, but do we practically realize what this means?

Then again, unless more of our senses than one are brought to bear upon a matter under consideration, we can scarcely form a true opinion upon our subject.

Take something which greets our vision for the first time. We know not what it is; we can see it, it is true, but we have to bring in the aid of other senses before we can arrive at a correct judgment; and even then, our judgment being the result of comparison, and also of experimental contact of substances with our senses—so to speak—opinions

which are formed must, to a certain extent, be modified by the amount of other experience to which our nerve centres have been previously subjected.

Take two experts; give to each one a sphere composed of lead and tin. Upon asking them what substance they were handling they might probably guess, perhaps not; they would poise it in their hands, look at it, smell it, try to cut it, perhaps, examine its metallic lustre, and it would be very odd indeed if they could agree as to the composition of the alloy, unless settled by an assay upon the balance.

Has it ever occurred to the reader that such processes as these go on in Microscopy, and that it is necessary to carefully study the organ of vision in order to gain a true insight into the object presented to us? On reference to plate 1, it will be seen that the eye is a nearly spherical ball, capable of many movements in its socket. It possesses an outer translucent covering called the sclerotic coat, or simply sclerotica, which may be seen at S. This is thick, horny, and opaque, except in its anterior portion.

This sclerotic coat envelopes about $\frac{5}{6}$ of the eyeball, and in common parlance is called the white of the eye.

The anterior transparent portion is called the cornea, and has the shape of a very convex watch glass. It is through this membrane that the light passes to the interior of the eye. The cornea and the interior portion of the sclerotica are covered with a mucous membrane.

Behind the cornea is a diaphragm of annular form called the iris; it is coloured and opaque, the circular aperture in its centre, *p* being called the pupil.

The iris, *i*, serves the purpose of regulating the admission of light; it varies in colour in different individuals, and is the part referred to when we speak of the colour of a person's eye.

Behind the pupil is the crystalline lens, *l*, having a much greater convexity at its posterior surface than at the anterior.

The large posterior chamber is lined by the choroid coat, and this choroid has in front of it a delicate membrane called the retina.

The choroid coat consists of a highly vascular membrane containing pigment cells, filled with an intense black mucus, called the pigmentum nigrum.

The cavity behind the cornea is filled with a liquid called the aqueous humour, having a refractive index approaching that of 1.3366, while the larger cavity is filled with a transparent jelly, called the vitreous humour, possessing a refractive index of 1.3379, enclosed in a very thin, transparent sac, called the hyaloid membrane.

I have now described the principal apparatus of the eye, and may take some of the parts in detail.

The crystalline lens is built up of layers, increasing in density inwards, the effect of which is to diminish spherical aberration. This lens is enclosed in a transparent capsule, held in position by an elastic membrane. It can be changed in shape by means of a delicate muscular arrangement to adapt its focus for near or distant objects.

As glass lenses of varying curves have different focal lengths, so by altering the curves of the crystalline lens we are able to see objects distinctly which are situated in several focal planes.

The reader may have noticed that there is a near point at which objects can be seen most distinctly; this point varies in individuals, but averages from 8 to 10 inches. As we move farther away from the object, although diminished in size, it may be seen more easily, and with less effort.

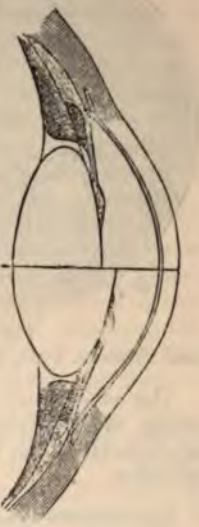


Fig. 1.

It would appear, then, that all objects are rendered apparently larger, as they continue to approach the eye, but a limit is soon found to this, as at a distance of six inches distinct and easy vision is not possible (except in very abnormal cases).

The reason of this is well-known—the anterior focal point of a convex lens when shortened lengthens the posterior conjugate focus, so that when an object is brought too near the eye the image of it is projected behind the retina, and the crystalline lens cannot accommodate itself to such extremes. But we know that objects can be seen distinctly at great distances apart, and it may be useful to demonstrate how this is brought about.

The figure (1) represents a cross section of the crystalline lens. The real mechanism of accommodation has been much disputed, but the results, as observed, are, that the curvatures of the crystalline lens are altered as the observer adapts his eye to near or remote vision; increase of curvature, of course, shortening the focal length of the crystalline lens, and being better adapted for near vision, while the shallower curve is necessary for the distant view of remote objects. Helmholtz has shown that the radius of curvature of the anterior surface of the crystalline lens may be varied by means of the muscular arrangement, from 6 to 10 millimetres.

Fig. 2.

We may now cast another glance at the iris. This apparatus is really a continuation of the choroid tunic which lies between the sclerotica and the retina: it ends in front, in what are called ciliary processes, as shewn in Fig. 2. The small muscular ring surrounding the pupil is called the sphincter muscle.

Now, the principal use of the choroid tunic, or rather the pigmentum nigrum which it contains is to absorb those rays of light which have passed through the transparent retina, preventing their reflection, which would interfere with the distinctness of the image.

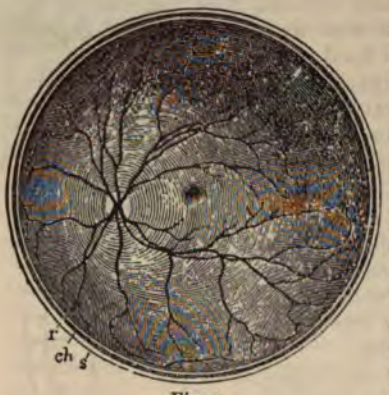


Fig. 3.

By referring to Fig. 3, after Henle, it will be seen that the chloride tunic, the retina, and sclerotica form the three outside rings, while the centre is ramified by nerve filaments and blood-vessels. (Fig. 3.)

These nerve filaments and blood-vessels lie in the retina, which really forms a continuation and extension of the optic nerve; it touches the outer circumference of the iris at the front, and lies open as a cup-shaped disc in the interior of the eye; it receives the rays of light which have passed in turn through the cornea, aqueous humour, crystalline lens, and vitreous humour, and forms a picture at the focus of these.

The nerve fibres of the retina are excited probably by a product of the action of the light picture upon the visual purple, and the irritations are transmitted to the brain by the optic nerve, producing the sensation of vision.

The picture produced upon the retina has been compared with that produced by a photographic lens upon a screen or ground glass; but it will be seen that the instances are not strictly parallel.

In the eye the rays falling upon the cornea do not again encounter air, the picture is formed *in* the highly refractive substance, while in the photographic image air intervenes between the screen and the lens, and between the lenses themselves.

Then, again, the adaptation of the eye to various distances is obtained by a process so dissimilar to that of the lens in the camera, that it is well no comparison should be instituted.

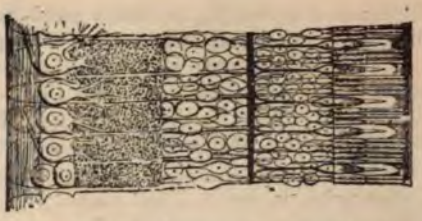
The retina has been previously described as a delicate membrane lining the choroid tunic, inside the sclerotica.

Now, if we make a section of the retina, we shall probably find its structure very similar to the diagram. (Fig. 4.) I say probably, as I have never met with sections which displayed the structure so well as Max Schultze has indicated. He has described the various layers which you see before you, as follows:—

Starting from the junction of the retina with the vitreous humour, we have—

The layer of nerve fibres .....*a*.	The outer granular layer .....*f*.
The layer of nerve cells..... .. ..*b*.	A second fine membrane ....*g*.
The granular layer.....*c*.	The layer of rods and cones ....*h*.
The inner granular layer .....*d*.	Pigmentum nigrum of the choroid .....*i*.
The intermediate layer .....*e*.	

The retina is the terminal organ of vision, all the apparatus in front of it being merely for the purpose of securing that an accurate image should be focussed upon it. As to how the luminous impres-ions yield to us such a definite idea of things is a question still under consideration, many have tried to solve it, but I am not sure whether we are any nearer the mark than those philosophers who lived 2,000 years ago.



a. *b.* *c.* *d.* *e.* *f.* *g.* *h.* *i.*

Fig. 4.

There are several curious properties inherent in the retina. By means of the ophthalmoscope may be seen a point, a little out of the centre, where the optic nerve enters the eye. This spot is totally blind, it cannot perceive a trace of light, and if the image of an object falls upon this blind spot, that object is totally invisible. It is at this spot also where the blood-vessels enter the eye, and ramify through nearly the whole of the surface layers of the retina.

In the centre of the figure (3) you will see also a dark shaded portion practically free from blood-vessels. It is a round, yellowish, elevated spot, about $\frac{1}{20}$th of an inch in diameter, and it is here that the sense of vision is most perfect. It is called the yellow spot of Sœmmering; it is not covered by the fibrous part of the retina, but a layer of closely-set cells passes over it, and in its centre is a minute depression called the *fovea centralis.* (Plate *f.*)

In the above description points only have been touched which directly bear on good or defective vision. On the other hand, enough has been advanced to show that this organ is liable to imperfections which may, and are, extremely liable to modify all our observations made over the tube of the microscope.

In order to produce a picture upon a screen, a lens is not absolutely necessary, if a diaphragm, perforated with a series of holes, be placed in front of the electric lamp, the screen will be decorated with as many images of the carbons as there were holes in the diaphragm; but another illustration will perhaps render this more evident. A small hole pierced in the shutter

Fig. 5.

of a darkened room (Fig. 5) allows of the passage of rays from a well

illuminated landscape, so that a small but inverted image is cast upon the screen ; the further the screen is placed away from the aperture the larger will the image be, though less distinct, and *vice versa*. The picture produced is not so good as that formed by a lens, it is dark and somewhat confused at the margin, and if the aperture is enlarged, there is still greater confusion, until the image is finally lost.

Now, if we take an ordinary lens of glass and attempt to produce a picture with it, we find the centre alone is plainly visible—the lens is afflicted with what is termed *spherical aberration*, that is, the rays from its periphery are brought to a focus in a different *plane* to those occupying a central position.

Now, this fault may be illustrated by Fig 6.

Fig. 6.

But although it is so easily shown in a diagram, a small amount of spherical aberration is not so easily detected by the student. It appears as a haze or fog of light over the object.

In the human eye this defect is not observable to any great degree, as the peripheral or more strongly refracting rays are cut off by the iris. Then, again, the curvature of the cornea is ellipsoidal rather than circular, so that the rays farthest from the axis are least deviated, while the two curves of the crystalline lens correct, so to speak, the one the other ; and lastly, this lens is of such construction that its refractive power diminishes from the centre to the circumference.

Another defect in the eye is due to the different meridians having dissimilar degrees of curvature.

If a set of concentric circles be observed with one eye, they are seldom all distinct at the same time, and there is produced a kind of Maltese-cross effect, not perceivable, perhaps, in many instances with large circles, but noticeable when drawn to such a size that the outer one is about two inches in diameter. (Fig. 7.)

Fig. 7.

This defect is called astigmatism, and known to oculists as a common cause of headaches. Spasm of the focussing apparatus may derange the sphericity of the eye, and so affect vision. Strained vision is very subject to this. On the other hand, the same apparatus may be paralysed, and ordinary vision deficient, whilst the focussing of the microscope might possibly correct it.

Astigmatism has injuriously affected painters; Turner for instance, whose later pictures are discovered to be slightly distorted, in consequence of the power of accommodation or self-correction having been lost from age.

In microscopic drawing, as with the camera lucida, the perspective may be misrepresented, in consequence of astigmatism, and thus endless disputes may arise even among the most careful observers.

We have now to deal with errors of refrangibility, and it will probably have been assumed that the eye apparatus is entirely corrected for colour. This is not the case, however, except when an object is in exact focus, and the reason that the error due to refrangibility remains practically unnoticed is that the distance between the focal point of the red and violet rays is extremely small. The error due to refrangibility may be noticed by means of the concentric circles already referred to; by bright daylight adjust the eyes to some object twelve inches away, and without moving the eye insert at a distance of four inches a card inscribed with black circles, when a yellow and blue colouring will be plainly discerned.

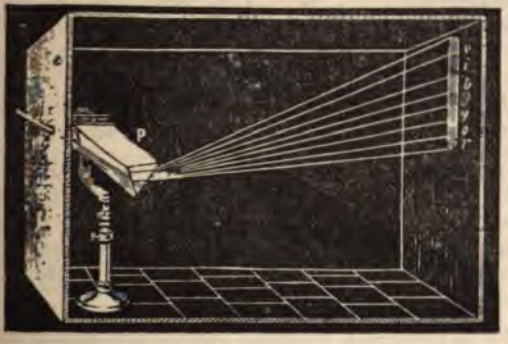


Fig. 8.

In order that the reader may thoroughly understand the error of refrangibility, the picture afforded by the passage of a solar ray through a prism of glass may be thrown upon a screen, the rays are deflected unequally, the red least and the violet most, as in Fig. 8.

It may be advisable here to state that the degree of dispersion of the rays of white light depends upon the medium through which the ray passes, and this amount of dispersion is measured by the distance of the most prominent dark lines in the spectrum from each other. The diamond disperses much less than crown glass, while the deflection of the ray is greater; but this is a subject beyond the scope of the present essay.

Now, beside these errors, there are others to which the microscopist should devote special attention; they are caused by small opaque particles existing in the transparent media of the eye-ball. These cast their shadow on the retina, and produce images which appear to exist outside the eye. These extra-retinal images often appear as globules, bacteriod-shaped bodies, or strings of minute pearls, and may be studied by directing the eye to a sheet of strongly illuminated opal glass, through a small aperture made with a fine needle in a piece of thin blackened cardboard. (Fig. 9.)

When the microscope is used in a vertical position, these globules often gravitate to the centre of the cornea, and even after prolonged use of the inclined tube an observer may often be perplexed by the layer of mucus, or a lachrymal discharge covering the surface of the cornea.

Just a few words as to colour perception. Colour is a special sensation excited in the retina by rays of a definite wave length, and the reason why certain objects are presented to our view with colour is that when white light falls upon a given surface, some is absorbed, the remainder being reflected. If the green rays are reflected, then the object appears green, and if the red rays are alone reflected, then the object will be red.

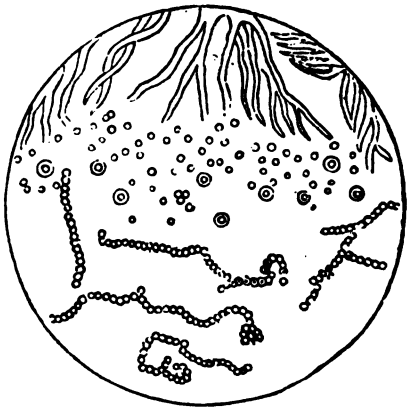


Fig. 9.

The generally accepted theory of colour perception is based on the assumption that three kinds of nerve fibres exist in the retina, the excitation of which produces sensations of red, green, and violet, and that modifications of these three sensations yield all intermediate tints.

This theory will explain some of the phenomena of colour blindness—if the nerve fibres which should give their special sensation are paralysed, or are wanting, the sensation only of the complementary tint will be transmitted with all the defects of the eye. It must not be forgotten that many phenomena consist more in errors of judgment than in absolute error of form or sensation.

Now in regard to errors of judgment, we must admit that all our estimations are made by comparison. In magnitude we are guided by the size of the retinal image as determined by the visual angle—for position we must have some starting point; and as for distance, every one knows how delusive an inexperienced estimate of this is. At sea, a landsman could not judge of the distance of a passing vessel to a few miles, nor could we form any accurate idea of the size of any object emitting practically parallel rays unless we had something to compare it with.

We now come to a point which has been much disputed in the study of microscopy—binocular vision.

The two eyes move together as a system, so that we direct the two lines of regard to the same point in space and consequently see but a single image; but it is possible to see two—if one eye be displaced a little with the finger two images are seen, while if the other be displaced to a corresponding degree the one image is restored.

The value of binocular vision may be easily ascertained by experiment. When a picture is presented to the retina of each eye, the compound picture is much brighter than when one retina only is employed.

To each point of the retina of one eye there is a corresponding point in the retina of the other, and impressions produced on one of these points are in ordinary circumstances indistinguishable from a similar impression produced on the other.

When both retinæ are similarly impressed, the general effect is that the impressions are more intense than when one eye only is employed; and we also get a perception of relief, that is of form in its three dimensions.

Take two A eyepieces and look through them to the sky, so that two distinct circles are seen; now bring them together so that one circle overlaps the other, when this overlapping bi-convex portion will be found double the brightness of the remaining portions of the circles.

We are indebted to stereoscopic vision for the perception of relief or form in three dimensions, which occurs when the images falling upon the corresponding points of the two retinæ are not exactly similar. In looking at an object with both eyes the rays do not run parallel from one side of the object to the eye on that side, but the right eye centres itself to the left side of the object and *vice-versa*. This may readily be seen by holding up a finger between our eyes and the wall, and looking at the latter. Two fingers may be seen projected on the wall, one of these is seen by the right eye and the other by the left; but our visual impressions do not inform us which picture is formed by either eye in particular. Now, while steadfastly looking at the wall, close the right eye and the left finger will disappear, while on shutting the left eye, the right finger is rendered invisible.

When two similar pictures are presented to the eyes, the impression is more vigorous and looked at with greater ease than when one eye only is employed; vision in this case is called pseudoscopic.

Binocular vision should be employed wherever practicable; it will be found much less trying to the eyes than monocular efforts.

I have now mentioned the leading features of the human eye, and shown that it is extremely liable to imperfection, and, being so, strict attention to details is demanded from the microscopist.

Fig. 10.

Now, although the human eye is such a wonderful instrument, there are many problems it is unable to solve without

extraneous help. Take, for example, the bunt of wheat, *Tilletia caries* (Fig. 10). With the unaided eye, you will be able to discern nothing more than a black dust, the various details having to be made out by other means. Then again, with objects so minute as the diatom, *Amphipleura pellucida* (Fig. 11), the object itself is almost invisible to the unassisted eye, to say nothing of the beautiful carvings with which the valves are embellished, and which exact for their elucidation the most perfect lenses with which we are acquainted, and the most accurate manipulation of the illumination. You may, indeed, see the contour of many forms of diatoms without extra optical assistance than that afforded us by nature, but not much more than this, as if the eye is approached too closely the picture falls behind the retina and is lost.

Fig. 11.

If we take a very much enlarged picture of the diatom, *Pleurosigma angulatum*, it illustrates in a remarkable manner how errors of observation are likely to creep in. It is hard to believe at first that the white circles which are seen are not hexagons, but are in fact true circles, which close investigation will prove.

I have already mentioned the fact that starting with the distance of most distinct vision, continued approach to the eye finally renders the object invisible, the rays being thrown *behind* the retina, the mechanism of accommodation being insufficient to produce a curve deep enough to bring the picture to a short conjugate focus.

This can, however, be done by interposing a lens or lenses between the object and the cornea, so that a *virtual* image of the object is seen. These lenses form either a simple, or a compound microscope.

THE PREPARATION OF ANIMAL TISSUES.

Most animal tissues require hardening; a few, such as bone and tooth, require softening, whilst certain structures are either destroyed, or the features we wish to see distorted, by either process, so that we have to examine them in media which we call indifferent, such as ¾% salt solution, etc. Besides the above, we have special operations, such as injection of blood-vessels, rubbing down of tooth, bone, and so forth.

We will first describe the hardening processes, prefacing our remarks by giving a few general directions. The requisite tissues have to be obtained from the newt, frog, pig, sheep, calf, ox, rabbit, cat, dog, horse, etc. We have further to requisition the water-beetle, crab, salt water mussel, skate, and tadpole. Further, we have to procure newly-born animals and fœtuses.

In hardening tissues from the above, as a general rule we must secure *penetration* of the liquid by placing the tissues in abundance of liquid—100 times their bulk or more, and dividing the tissues into sufficiently small pieces. The latter object is attained by allowing a quarter of an inch, and not more, for the fluid to penetrate. Thus, in placing a solid organ, such as liver or kidney, in hardening fluid we should cut it into half-inch cubes, then the deepest part of the cube would be a quarter of an inch from the surface. For membranes and organs which have no part a quarter of an inch from the surface such as omentum, trachea, etc., we divide the parts for other objects, it may be, but not to reduce them for penetration of the hardening liquid.

The tissue must be as *fresh* as possible. Parts which have to be treated by the silver or gold processes must be taken within half an hour after death, or the parts affected by our re-agents will have ceased to be susceptible. The time of year also has much to do with this, summer and hot weather being a worse time than the cold weather of winter. No tissue can be placed in its preservative medium too early. All foreign matter such as contents of bowel, etc., must be removed by ¾% salt solution. After a fluid has become fouled by the tissues placed in it, it should be changed. All tissues placed in chromic acid solutions must be examined daily after a few days to prevent them becoming brittle and spoiled. Chromic acid is an excellent medium, but it renders the tissue brittle, and spoils it if kept a day or two too long in it. Then, again, different tissues in the same medium require separate testing. Some may be hard enough and require immediate removal, whilst others may remain longer. No tissue should be allowed to remain until it is quite hard in the chromium preparations, if we wish to finish the hardening in spirit, as is commonly the case, but should be removed when it has become *tough*, and has still plenty of elasticity. In changing a tissue to complete its hardening in spirit, we must first of all wash away all its former hardening medium by soaking it in plain cold water, frequently changing the water during the twelve or twenty-four hours we keep it there, so that

the last washings may be colourless. We must not put tissues thus prepared direct into spirit, but first place them in methylated spirit and water, half and half, for twenty-four hours, then in meth: spirit pure. In some cases such as testes, brain, tonsil, etc., we require to transfer them from the common spirit to absolute alcohol, before making sections. The object of hardening is to shrink the tissues, but in shrinking them we have to shrink them uniformly, and therefore, gradually; therefore, a fluid that would harden and not penetrate, would harden and shrink the tissues nearest the surface, and perhaps allow the interior to soften and rot. As a rule, we must commence with weaker, and advance to stronger, solutions, for the purpose of penetration, and even shrinking. Not only so, but we often have to commence with a fluid remarkable for its penetration—such as Müller's Fluid—which penetrates to great depths, but is slow in its hardening capacity, requiring weeks in doing so, but frequently not hardening some tissues, sufficiently, at all. We must keep a small book, with copious notes as to the kind of animal, the tissue, the date and manner of its death, the nature of the fluids it is in, or has already been in, dates of changing, or of substituting fluids, etc. Each bottle should be labelled with a letter or number, or what is better in some cases, a letter and number, any sufficient mark, in fact, which may have its corresponding mark in our note book. Mere labelling of the bottle, and making notes upon it, will not do for many reasons. For extra distinction, we may either wrap the tissue in linen, and tie a label to the linen, or we may place special tissues, easily distinguished loose, and in the same medium.

With a further remark, that no hard and fast line can be drawn in the matter of *time* any substance may require to be in a fluid before its transference to another fluid, but in most cases the operator must use his judgment, which, after all, requires some experience, we will now give a list of tissues arranged under the hardening fluids. It will be found that the *same* tissue will appear under more than one preparing medium in many cases, because the various elements in a tissue may not be all demonstrated by treatment in any one fluid; thus, if we wish to shew the cornea corpuscles and nerves, we treat a cornea with gold, whereas, if we wish to see its cell spaces, we treat it with silver. So that the same tissue, cornea, will be found under gold process and silver process: the same obtaining between absolute alcohol and the chromium preparations, and still further between the sarcous substance preparations themselves.

Chromic Acid.

We make a 1% solution with common water, and reduce it to a fourth, sixth, or any other percentage solution we require.

Bone deprived of its muscles, but not of its periosteum, is steeped in very weak solution, then in stronger solution of chromic acid, commencing with a tenth per cent. and rising to a half, through a period of ten days. It is then decalcified by steeping in chromic and nitric fluid until a needle can be pushed through it. Then it is washed and transferred to spirit. A tooth may be treated the same

MUSCLE.—A bit of muscle from an animal that has been dead a few hours is steeped in a small quantity of a ½ % solution for a week. The cleavage of its sarcous substance is shown by teasing a bit in glycerine.

NERVE.—Harden a piece of meta carpal nerve of a horse, or sciatic nerve of a smaller animal, for ten days in a ½ % solution. Stain a bit in logwood, then tease it in glycerine thoroughly. This shows its connective tissue.

CHROMIC ACID AND SPIRIT.

Mix one part of ½ % solution of chromic acid with two parts of methylated spirit. This should be done when required for use.

The following tissues may be placed in this, then transferred to spirit. The time required must be judged according to directions already given :—

The whole of one cornea, to show stratified epithelium ; a piece of small intestine, to show non-striped muscle ; heart and pericardium of a small animal ; small arteries and capillaries from the brain of a sheep, after scraping away the brain-substance ; middle-sized artery—such as metacarpal of the horse ; trachea and lungs. These are gently injected, then immersed in the fluid.

The lips, tongue, salivary glands, tonsils, œsophagus (distended and tied), stomach (after gently washing away its contents with ¾ % salt solution), small and large intestines, liver in half-inch cubes, ureter and bladder (distended), ovary, fallopian tubes, uterus (distended per vaginam) may be placed in the solution.

Besides the above, which may be taken from either a dog, cat, or guinea pig, the following should be obtained :—The thymus gland of an infant ; skin of scalp, finger and palm of hand, sole of foot from the human subject ; also a nail. The eye of an ox divided transversely just behind the cornea for the ciliary muscle, sclerotic, cornea, and iris ; also the choroid and retina. Of course both halves have to be used. The prostate gland and penis of a guinea pig. The cervix uteri of a cow. Mammary gland of an animal near the full period of gestation. The placenta of a cat, or guinea pig. The umbilical cord, which must be cut into pieces an inch long, and hardened for two days in Müller's fluid before being placed in the present medium.

With all the above the tissues must *daily* be examined, after the third day, and each transferred after it has become *tough*. Moreover the fluid should be changed after the first twenty-four hours, whether changed afterwards or not. When many different tissues are in the same jar, the quantity of fluid must be such that the upper surface of the tissues extends half way up the entire fluid, that is to say, the stratum of tissues and stratum of clean fluid over them had better be of equal depth.

BICHROMATE OF POTASS.

Make a 2 % solution of bichromate of potass, with ordinary water.

Meso-rectum of Cat. Pin this out on cork, and float it cork upwards on the solution for seven days.

Liver. After injecting the portal vein with blue gelatine mass, and the hepatic artery with carmine gelatine mass, the liver may be hardened in the solution to toughness, and, of course, finished in alcohol.

Spinal Cord of Ox. Pieces an inch long may be placed in the solution—frequently changed—from three to five weeks.

Spinal Cord of Ox, Horse, or Sheep. If pieces about an eighth of an inch long be macerated two or three days in an eighth per cent. of the solution, the anterior horn of the spinal cord snipped out with scissors, and teased in carmine solution, then pressed with a cover glass, using Farrant's medium, will show the isolated multipolar nerve cells very beautifully. We may isolate the sympathetic nerve cells of the frog in the same manner.

Cornea of Cat, Rabbit, or Guinea-pig should also be hardened in a two per cent. solution for ten days. The lens in a one per cent. solution for one week.

Ovaries of the Cow in very small pieces should be macerated in very dilute solution to isolate the large, branched pigmented cells of the Corpora lutea.

Ammonium Bichromate.

A two per cent. solution made with ordinary water may be employed.

This solution is preferred by many to the potass salt solution. It is used in the same way. Columnar epithelium may be prepared as a permanent specimens by placing a piece of fresh intestine of dog, cat, rabbit, etc., in a one per cent. solution for two days; then steeping an hour or two in water and scraping off the epithelium and staining. The cells have to be separated with a needle, and may be mounted in Farrant's medium, or in glycerine jelly.

Chromate of Ammonium.

A five per cent. solution is used.

If a newt's liver (in small pieces) and pieces of the small intestines be placed in the solution for forty-eight hours, the liver cells and columnar epithelium may be obtained, as in the case above-mentioned. The goblet cells of Klein can be beautifully preserved in glycerine jelly in this way. The mesentery of the newt may be placed in the solution at the same time, and taken out after twenty-four hours. This shows the non-striated muscle fibre beautifully. The isolated gastric glands of a small mammal may be obtained in the same way by placing bits of the fresh mucous membrane for three days in the solution. The testes of the newt should be placed in the solution for twenty-four hours, then cut into, and their contents squeezed out on to a slide. The spermatozoa are thus obtained as a permanent preparation.

Another important use of the above solution has been pointed out by Heidenhain. If small pieces of kidney be placed in the solution for forty-eight hours (the cortex should be chosen,) the preparation shews the cells of the uriniferous tubules and their peculiarities clearly as no other method, probably, can shew them.

Müller's Fluid.

This is made by dissolving 25 grms. of Potas bichrom ; and 10 grms. Sodae Sulph. in 1000 c.c. of water.

Müller's fluid has great penetrating power. It hardens slowly, taking it may be five to seven weeks. It is useful as a commencing agent to be followed by another of greater shrinking power, such as chromic acid and spirit solution, common alcohol, etc. Very even shrinking may thus be obtained. For example if we cut out the fresh nasal septum and place it for two days in Müller's fluid, then for a week in Chromic acid and spirit solution, afterwards in weak, then in pure methylated spirit, we get the olfactory epithelium in excellent preservation.

After the above explanation the reader will have no difficulty with the following delicate structures namely :—Developing tooth, the adenoid tissue of lymphatic gland, spleen, thyroid gland, supra-renal-capsules (of the horse, by preference) sympathetic ganglion, olfactory epithelium, cochlea, testis, epididymis and vas-deferens, ovary, human placenta, etc.

Müller's Fluid and Spirit.

Take three parts of Müller's fluid and add to it one part of methylated spirit and keep it in a dark place. The mixture should be made only as required.

The above is especially useful for the central nervous system. The removal of the brain and spinal cord without injury may here be described. Immediately after death the skin is removed, or at least the skin over the back and neck. Then we separate the neck and head from the trunk about the middle of the neck. Next we clear away the muscles on each side of the vertebral spines and clip away every spine as close as possible with scissors or still stronger shears, such as bone forceps. Next with ordinary forceps we grasp the laminæ, which cover over the spinal cord, one lamina at a time, and break it outwards by inserting one blade of the forceps *within* the neural canal, the other on the upper surface of the lamina. We advance down the spine breaking the laminæ outwards, right and left till all the cord with its membranes is exposed. We deal with the head part likewise by breaking off, bit by bit, the top of the skull, inserting one blade into the interior, of course, after clearings away the skin and muscles.

The *spinal cord and membranes* of a cat, dog, or rabbit should be carefully got out, and suspended in a deep, narrow vessel. The fluid should be changed at the end of 24 hours, then after a week. At the end of this time, divide into pieces an inch long, and continue the hardening for another week or two, as may be required. It may be replaced by a two% solution of bichromate of ammonium for two weeks, and the pieces preserved in spirit, or by Hamilton's method (choral hydrate, 12 grains ; water, 1 ounce). The cerebellum, cerebrum, and, of course, the medulla oblongata may likewise be hardened. In the case of the two former we have to divide into suitable pieces for the sake of perfect penetration.

The *tendo achilles* of the calf, and the metacarpal nerve of the horse, each in inch long pieces ; also the posterior half of the eye-ball of a pig (for the retina) may be hardened with advantage in this fluid.

Absolute Alcohol.

This should have a specific gravity of 0·795. It hardens in 24 hours, with much shrinking. It is used for secretory glands notably the pancreas, which must be placed direct in it, or the gland may spoil by partial self digestion. Therefore we may place the salivary glands, the lachrymal glands, pieces from both ends of the stomach for the gastric glands, the pancreas in pieces, etc., in it at once.

If we inject the lymphatic gland of a horse, ox, or smaller quadruped with two % Prussian Blue fluid by Klein's method,—introduce a glass pipette filled with the fluid into a lacteal near a mesenteric gland and blow the solution into it,—then place the gland in absolute alcohol, we have the lymph sinuses well injected. The muscle structure of the beetle or crab is shewn well by placing a beetle, or the amputated limb of a crab, for a week in absolute alcohol. A bit of muscle is scooped out, stained and mounted, after being teased with needles. Non-striped muscle may be demonstrated thus:—Kill a small animal and wash out a length of the small intestine with salt solution: distend it with abs: al: and tie both ends; then suspend it in the alcohol for twelve hours. With a pair of blunt-pointed forceps we can now tear off strips from the outer surface, which will include the longitudinal muscle structure: stain them and mount in balsam.

Picric Acid.

Make a cold saturated watery solution. Small pieces of tissue harden in this in from twelve to forty-eight hours. It is excellent for decalcifying fœtal bones, which may be left in it, and tested from time to time with a needle, which ought to be pushed easily through them. No time can be specified, in some cases perhaps weeks may be required. Prepare the varieties of cartilage with this solution. The aorta (pieces) of a large animal (horse, or ox), also a cornea to shew its fibrous tissue: the thymus gland of an infant: also a lymphatic gland may all be well prepared by remaining in this solution twenty-four, thirty-six, or perhaps forty-eight hours. The intracellular plexus of fibrils may be well shewn, thus:—Keep a newt in a little water for three, four, or five days, without changing the water. Its outer layer of cuticular epithelium is shed as a cast of the entire animal. Place the film in the solution twenty-four hours, then wash in plain water till no colour is given off, and preserve in common alcohol till required. A snip of this, stained in picro-carmine, and mounted in Farrant's medium, shews the above-named structure beautifully. The fibres of white fibrous tissue may be shewn to advantage, thus:—tear off fine strips of tendo achilles of an animal, and place them for twenty-four hours in the solution. By teasing a little piece in water, the white fibres, held together by cement substance, are well seen.

Osmic Acid.

This is purchased as a 1% solution and diluted with distilled water to a half, a quarter per cent. solution, etc., as required. It is a hardening agent which also stains fatty matter and is, therefore, useful in blackening the medullary nerve fibres. It is very poisonous, very expensive, and soon spoiled by exposure to light. It must be kept in a well

stoppered bottle, and this must be pasted round with black paper so that not a particle of light may be admitted. Substances placed in it harden in from four to thirty-six hours, and may be prepared and mounted in either glycerine, glycerine jelly, or Farrant's medium. Very little bits, not larger than half a grain of wheat, are to be used of any tissue, and the bottle they are placed in must also be very small, the little short glass tubes in which homeopaths keep their least pilules answer well.

With the above cautions and directions we may enumerate the tissues which are treated with advantage by osmic acid. In a 1% solution treat costal cartilage of a kitten, puppy or young rabbit, rat or guinea pig, for twelve hours: a bit of the sciatic nerve of the frog for ten minutes: the non-medullated nerve running in the wall of the splenic vein of an ox, also a bit of human placenta. A 1% solution may be injected into a testis with a hypodermic syringe, and the whole organ may then be placed in alcohol. The following are best treated with a half per cent. solution, namely:—liver cells of a dog or small animal (the liver is cut across and the cut surface scraped) are placed in the solution for an hour. Areolar tissue is demonstrated by injecting a half per cent. solution into the groin of a puppy or kitten *immediately* after death. A bulla is thus formed, which must be snipped out with scissors, spread on a slide and stained with logwood then covered. A half per cent. solution may be forcibly injected into the anterior horn of a fresh spinal cord. The part is cut out, macerated for two days in dilute alcohol, and the multipolar cells isolated by teasing, after staining with carmine. A quarter per cent. solution (the strength most frequently employed) may be used for shewing mucous tissue; thus:—inject the axilla, or groin, of a very young embryo, as described above. Pieces the size of a pea of the following may be hardened in a quarter per cent. solution: Submaxillary gland, fresh pancreas, pieces from each end of the stomach, small intestines, supra-renal capsules of the horse, human skin. Place a piece of the anterior horn of a fresh spinal cord for 10 days in a tenth per cent. solution, then, after washing away the greyish deposit, place it in an equal quantity of glycerine and water for a fortnight; stain with weak magenta solution, tease in glycerine, and cover (Stirling).

MISCELLANEOUS PREPARATIONS.

Salt Solution.

This is made as a three-quarter per cent. solution, that is, ·75 grm. in 100 cc. of water. It is used for washing away foreign matter from organs before immersing them in other fluids, and for examining fresh tissues. In so using it, we place a very small drop on a slide, then immerse in this a minute piece of the structure we wish to examine, and tease it out with needles; then put over it a cover glass.

The following are to be examined:—Fresh columnar epithelium for the small intestines: ciliated epithelium scraped from the roof of the frog's mouth: ciliary motion may be seen in the yellowish coloured gills of the common salt water mussel. Ligamentum nuchae of ox: subcutaneous connective tissue: adipose tissue: red marrow from a long bone: striped muscle best seen in sartorius of the frog: nerve fibre, sciatic of the frog: fresh pia mater: fresh spleen (the ox spleen by preference):

thymus gland: kidney: Gasserian ganglion: placenta: decidua, etc. may all be so treated.

Glycerine.

Margarine crystals may be obtained by steeping morsels of fat for twenty-four hours in glycerine. These post mortem products appear as delicate needles.

Water.

Squamous epithelium may be stained in magenta solution and examined in water. To obtain it, scrape the inside of the cheek with a blunt knife. The surface of the cheek may be also scraped and the scraping examined in water and afterwards irrigated with a five per cent. solution of liquor potassae.

Dilute Alcohol.

Mix two parts of water with one of rectified spirit. This is a useful dissociating solution recommended by Ranvier.

Olfactory, ciliated and transitional epithelium, may be prepared as permanent preparations thus:—Place a small piece of fresh trachea of a small quadruped for ciliated—a piece of fresh bladder for transitional—the head of a frog, with the nostrils slit up, for olfactory epithelium, in the solution for two days. The parts are then scraped and the scrapings stained and mounted in glycerine, or glycerine jelly. Stirling "fixes" the ciliated epithelium by placing the scraping in a 1 per cent. solution of osmic acid. Non-striped muscle may be obtained by taking out the bladder of a fresh-killed frog and distending it with the solution, then placing it for twenty-four hours in the solution. After brushing away the mucous membrane with a camel's hair-brush, we may stain the bladder in picro-carmine, and mount a piece in Farrant's medium. We may get isolated heart muscle fibres of the frog's and mammalian heart by following out the same principles. Purkinje's fibres can be obtained by snipping out bits of them (seen as fine transparent lines on the inner parts of the walls of the ventricles of the heart of oxen and sheep), and placing them in the solution for two days, etc. The villi of human placenta can be isolated by soaking a piece of placenta two days in the fluid.

Chromic Acid and Nitric Acid Solution.

This is made by adding 1 c.c. of strong nitric acid to every 100 c.c. of a half per cent. solution of chromic acid. It is used as a decalcifying agent for the most part.

Tooth, intervertebral disc with subjacent bone, articular cartilage with subjacent bone, costal cartilage of an old person, may each be placed in a large quantity of the fluid, which must be frequently changed, until the bony part is softened, which may be known by the needle test. The parts are washed free of the acids by frequent changes of water, then placed in weak, afterwards in strong, alcohol for preservation.

Permanent preparations of elastic tissue may be made thus:—Soak half-inch cubes of ligamentum nuchae of ox or horse in the fluid for a week; wash free of acids and preserve in spirit, and make transverse and longitudinal sections in the usual way. (Stirling).

SILVER NITRATE.

This reagent may either be treated of under the present heading of preparing by *hardening, softening*, etc., or under that of *staining*. We prefer the former for this reason :—All tissues for microscopic histology are prepared from the fresh subject, so are those treated by the silver and gold processes.

We use this reagent to darken the outlines of endothelial and cartilage cells. The cement substance between the cells absorbs our solution ; we then wash away the whole of the solution not so absorbed, and expose our tissue to diffuse daylight. We thus have the absorbed solution decomposed into an oxide of silver. Should we be so unfortunate as to allow the least silver solution to remain *upon* the tissue, our preparation is spoiled, or at least rendered unsightly by the black oxide granules of the decomposed silver nitrate spotting it all over. Again, we must wash the tissue, when possible, in distilled water before treating it with our silver solution to get rid of any chlorides there may be. After the slide is quite finished the less it is exposed to light the better, after we have induced brown or dark brown silver *lines*. The tissue must be taken within a few minutes after death.

Make a 1 per cent. solution with distilled water, taking care that the water is thoroughly distilled. We now keep this in a bottle coated ALL over with black paper. Stationers sell this paper ready made ; it is gummed on one side and has a dead black surface at the other. We then measure out the 1 per cent. solution, and further dilute to a half, a third, or a quarter per cent., as required.

Omentum of rabbit.—Kill a rabbit by bleeding. Remove the omentum and wash it in distilled water : place it in a $\frac{1}{4}$ per cent. solution ten minutes: wash in ordinary water *thoroughly :* place the whole in a saucer containing water, and expose to diffuse daylight till slightly brown. Small pieces are now stained with logwood and mounted in glycerine or glycerine jelly.

Septum Cysternae Lymphaticae magnae of a frog.—Kill a frog and immediately open the abdomen. Gently push on one side the stomach, bowels, etc., and pour distilled water on the part behind the stomach, when a delicate membrane floats up. Now pour a $\frac{1}{2}$ per cent. solution, drop by drop, over this till it becomes milky, and treat as above.

Lungs of a kitten, or puppy.—Distend these with a $\frac{1}{2}$ per cent. solution immediately after death, then ligature the trachea, and sink them in alcohol till required.

Tendon.—Tendons and their sheaths are covered by endothelium. Pinch the tail end off a recently killed mouse, and wash the fine tendon fibres thus drawn out in distilled water, and treat with a $\frac{1}{2}$ per cent.

solution. Take another set of these fibres, pencil off their endothelium with a camel hair brush dipped in distilled water, then silver. This shows the cell spaces in tendon.

Cornea.—The cell spaces of cornea may be shown by scraping away the epithelium from the anterior surface of the cornea of a pithed frog, then applying a 1 per cent. solution till greyish white. The cornea must be snipped at its edges, after being silvered, to make it lie flat on the slip. A rat's cornea may be likewise treated and stained with logwood.

Blood Vessels.—Kill a small quadruped by bleeding. Syringe out the blood vessels with distilled water; then inject a half per cent. solution. Use the spleen, mesentery, and intestines. The spleen must be hardened in alcohol, and sections cut, exposed to light, after staining with logwood, or not, as desired. This shows the endothelium of the venous sinuses. The intestines must be cleared of their contents by distilled water: exposed to light in a saucer containing water; a piece of small or large intestine is snipped out, laid on a slide with its mucous membrane upwards, which is gently scraped away, the muscular and serous coats remain, and are to be mounted in balsam.

Membranous Connective Tissue.—The omentum of an adult cat and rabbit, also that of a young rabbit, is to be treated like the omentum of rabbit, to shew the membranous connective tissue. The omentum of the young rabbit shews *developing* fat cells and blood-vessels. Some of the above ought to be stained with logwood.

Adenoid tissue.—With a hypodermic syringe inject a fresh lymphatic gland with a ¼ per cent. solution, and after placing it for twenty-four hours in alcohol, make sections. These are stained with logwood, and exposed to light till brownish.

Cement substance of non-striped muscle.—Wash out a small length of intestine of a rabbit with distilled water; then fill it with a ¼ per cent solution, and tie both ends: then place it in a ¼ per cent. solution for a quarter of an hour. Wash away all the silver, and cover over with water in a saucer, etc. Thin laminæ of the outer muscular fibres are to be stripped off with broad nibbed forceps. Mount some stained with logwood in balsam; others unstained, as desired. These also show the lymphatics.

Sciatic Nerve of a Frog.—To show Ranvier's crosses, kill a frog, and dissect out the sciatic nerve, wash it in distilled water, then tease a piece a line in length in a ¼ per cent. solution for five minutes. Wash it now thoroughly in water and tease it carefully in glycerine, cover, and expose to light till brownish. Mount a small length of an entire thickness of an intercostal nerve of a rat or mouse, to show its endothelial covering. Of course it is not to be teased, neither must it be other than gently washed, or the endothelium will be knocked off.

Lymphatics of the Diaphragm.—Expose the posterior (ventral) surface of a rabbit or guinea pig, immediately after death by bleeding, thus:—Ligature the gullet and the posterior vena cava, then remove all the abdominal viscera. Tie up by the hind legs, then with a brush dipped in distilled water brush away all the epithelium covering the centrum tendinum of the diaphragm. Wash silver solution over it, and treat as already explained. This silvers the endothelia lining the lymphatics.

CHLORIDE OF GOLD.

This salt is sold in small tubes weighing 15 grains. A two per cent. solution is to be made with very pure distilled water, and kept in a black bottle, like the silver solution. The operator must also provide himself with formic acid and two or three fresh lemons.

Development of Capillaries.—Snip off the tail of a half-grown tadpole, place it in a watch glass, and squeeze the juice from a fresh lemon over it and let it remain immersed for five or ten minutes; wash it in distilled water to remove all the juice, then steep it for half an hour in a 1 per cent solution. Wash away the surplus gold, then place in a mixture of one part of formic acid and three parts of water for twenty-four hours in a cool place and away from the light. The gold chloride will then be reduced.

The following are to be treated on exactly the same principles:—

A whole cornea.—That of a pig, dog, or cat, to show the nerves of the cornea, also the cornea corpuscles.

A piece of skin.—This should be taken from the snout of a pig or a mole, or both; also the soft part of a duck's bill. Sections show the nerves of skin.

Striped muscle.—The recti muscles of a rabbit's eye are to be taken, a strip cut off lengthwise. These show nerve terminations in striped muscle.

Muscle with its tendon.—Take a piece of the diaphragm of a rabbit: let the piece be part of the centrum tendinum with its attached muscle. Sections made in the long axis of the muscle fibres show the terminations of muscle in tendon. That is to say, the connecting links are shown.

Tendon from the tail of a mouse.—After treating a few leashes as above, snip off a piece a line in length: tease in glycerine and cover, or simply press the cover glass upon it till it flattens out. This preparation shows the relations of the cells and fibres in tendon.

Tail of rat.—After treating as above, with the exception that it must remain an hour in the gold solution, decalcify, by placing in chromic and nitric solution, then make transverse sections and stain.

Nerve Ganglia.—Treat the heart of a frog, in small pieces, as above; then, with a dissecting microscope, dissect out the nerves and ganglia. These are also found lying along the course of the abdominal aorta of the frog, and may be taken from here if preferred. The nerve ganglia of the bladder and ureter of a small mammal may likewise be thus demonstrated.

THE INJECTION OF BLOOD VESSELS.

Blood and lymphatic vessels are easier of demonstration when filled. The former are better filled with a fluid which becomes solid in ordinary temperatures. The vessels must be fully distended at the time and this

distention must remain. Gelatine forms the foundation of injection masses for the above purposes, because it can be liquified at blood heat and it solidifies in a little lower temperature; then, again, it is capable of being easily cut, and does not become brittle but remains tough and resisting though sufficiently soft. Colouring matter is added to distinguish the arteries from the veins, and these again from other channels, such as bile ducts, lymphatic vessels, &c. An imitation of the colour of the natural fluid the vessel contains is mostly preferred:—red for arteries, blue for veins, and so forth.

Red Mass.

This is made of gelatine coloured with carmine. There are several ways of making it, but whatever way may be chosen the *greatest care* must be exercised in making it a *neutral* or at least a *slightly acid* mass; because, if it be alkaline it will diffuse through the vessels and stain the adjacent tissues and render the preparation completely worthless. The mass had better be a little more than neutral, slightly acid, but if too acid granulation of the carmine takes place, and the fluid will not be driven into the arterioles, much less the capillaries. Parts injected by a carmine and gelatine mass must be immersed in equal parts of water, and meth. spirit, having 1 p.c. of acid in it.

Carter's Mass.—Dr. Carter directs the mass to be made as follows:—

Take of Carmine, 60 grains.
Strong ammonia, 120 minims.
Glacial acetic acid, 86 minims.
Solution of gelatine (glyc: 1 part water 6 parts) 2 oz.
Water, 1½ oz.

Dissolve the carmine in the ammonia and water with the aid of a gentle heat, and filter; add to this 1½ oz. of *hot* gelatine solution, and mix thoroughly. Add the acid to the remaining ½ oz. of gelatine solution, and drop this into the heated carmine mixture, with constant stirring.

Dr. Stirling's Mass:—

Take of Carmine, 60 grains.
Strong ammonia, 60 minims.
Glacial acetic acid, 80 minims (about).
Gelatine (Cox, or Coignets), 1 oz.
Water, q.s.

Soak the gelatine in water several hours: pour off the water which is not absorbed after the gelatine is completely swollen up, and melt it in a water bath. Then strain, while hot, through flannel, and make up the solution to 2 oz. Place the carmine in a mortar, and add to it the ammonia and 2 oz. of water, and leave it for twelve hours. Then filter, and add the acetic acid drop by drop, stirring all the while, until the ammonia is completely neutralised. As the ammonia becomes faint, the acid must be added very cautiously. As long as there is free

ammonia the fluid is *dull* red, but becomes a *florid, bright* colour the moment the ammonia is neutralised. Now mix the two solutions at a temperature of 40° C.

Dr. G. Sims Woodhead's Mass:—

Take of Carmine (pure), 4 parts, by weight.
Liq. ammonia, 8 parts, by measure.
Gelatine (Cox and Coignet's), 10 parts, by weight.
Distilled water, 100 parts, by measure.

Put the carmine in a mortar, and pour on the ammonia, when an almost black paste will be formed, if the carmine is pure; pour on the water, and set the solution aside to filter. Place the gelatine in a narrow glass jar, and add sufficient distilled water to cover it, and allow it to stand till the gelatine is thoroughly softened. Warm the carmine solution in a pan of water (kept nearly boiling on a gas jet or near the fire), and add the gelatine; stir thoroughly, and add a ten per cent. solution of acetic acid, drop by drop, until the alkalinity of the ammonia is neutralised, and the fluid even slightly acid. The point at which this takes place will be recognised by the *pungent odour* of the ammonia becoming gradually lost, and that of the acid substituted, and the fluid loses its bright carmine, transparent colour, and turns a dull brownish red.

We have given the above formulæ for making the same thing for several reasons. First of all, the operator will be struck by the contrast, which is so slight, but which does exist. We have given the three in their order, reckoned by their date of publication. There would be no reason whatever for not adhering rigidly to Carter's formula, but each operator thinks he sees a better way of neutralising the ammonia. With the exception of the change of colour test, we prefer Stirling's method, which, however, is greatly improved by making a diluted acetic acid solution, by adding the acid to a dram or two of water, when the pouring to or adding is more easily controlled. It will be noticed that Dr. Woodhead takes a ten per cent. solution.

Blue Mass.

This is made by adding soluble Prussian blue in place of the carmine.

Take of Soluble Prussian blue, 4 drams.
Gelatine, 4 oz.
Distilled water, 20 oz.

Treat the gelatine the same as in making the carmine mass, using half the water; then add the Prussian blue dissolved in the other half of the water, keeping both solutions hot, and constantly stirring whilst cooling is going on.

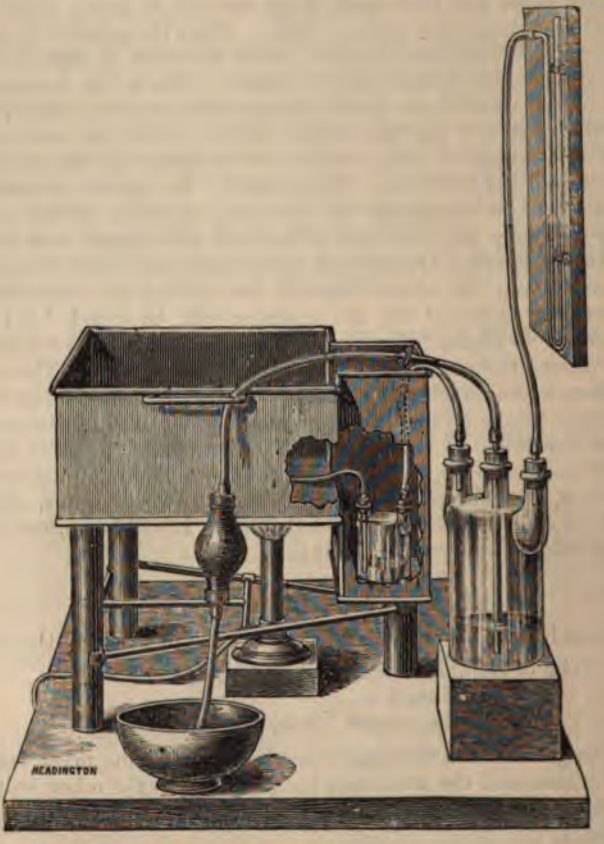
Injecting Apparatus.

The most usual way of injecting the blood-vessels is by means of the ordinary injection syringe. This requires a great amount of practice. The animal has to be kept in hot water; the mass has to be kept hot usually in a separate vessel, and time has to be allowed between each

syringeful, for the fluid to penetrate. Then, again, if air gets in on introducing the point of the syringe into the socket of the pipe that is tied into the artery, the fluid will not run at all.

The constant pressure apparatus of Ludwig, now that a simple and thoroughly efficient method of getting the constant pressure by means of an ordinary Higginson's syringe, as discovered and described by Fearnley, will take the place of the syringe, and of every other method in future.

No practice is required with this simple contrivance, beyond introducing and tying in the nozzle in the aorta. There is a bath, having a



shallow part for the animal to lie in, and a deeper part for the Woulff's bottle, containing the injection mass, to stand in. A large (40 ounce) Woulff's bottle, with three necks, is fitted with three perforated india-rubber stoppers. The middle stopper is perforated with a glass tube, which goes to the bottom of the bottle. Each of the others is perforated with a glass tube, the depth of the stopper only, and standing above the

stopper sufficiently to admit of a piece of indiarubber tubing (such as is used with infants' feeding bottles) being fixed upon it. The Woulff's bottle containing the mass, has two necks, fitted with indiarubber stoppers. One neck admits a piece of glass tube, which goes quite to the bottom of the bottle; the other admits a short piece of tube, the depth of the stopper only. The diagram shews all further detail. The apparatus is made by Messrs. Swift & Son, 81, Tottenham Court Road, London, W.C.

FEARNLEY'S CONSTANT PRESSURE APPARATUS.

The mercurial manometer allows five inches rise of the mercury in the ascending arm—therefore, five inches fall of the descending arm—though four inches will do.

To inject an animal—a rabbit, for instance,—proceed as follows:—Fill the bath with water, and heat the water with a Bunsen's burner to 100° Fah. or so. The Woulff's bottle containing the mass should be filled and thoroughly stoppered Then chloroform the rabbit and make an L shaped incision into the thorax, so as to expose the heart and aorta. This is done by cutting up the *middle line* of the sternum (breast-bone) as far as the root of the neck *nearly* then making a second incision at right angles to this, to the rabbit's left. A triangular flap is thus made, and the heart enclosed in the pericardium exposed. Having cut through the pericardium, seize the apex of the heart with a pair of forceps and snip it off, then the heart's apex appears as in A, Fig. 2. That is to say, the right and left ventricles are opened and the animal instantly bleeds to death.

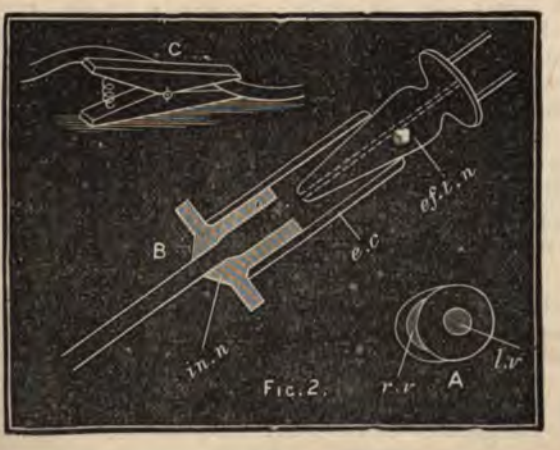


FIG. 2.

The opening in the right ventricle, leading to the pulmonary artery has a crescent shape or slit-like appearance; whilst the opening in the left ventricle, leading to the aorta, is round. Therefore, if we wish to

inject the entire arterial system, we insert our nozzle into the round hole, but if we wish to inject the pulmonary system only we choose the crescentic slit.

Either glass nozzles, or those shown in Fig. 2, are to be inserted into one or other of the two holes (usually the round one for injecting the entire arterial system with carmine and gelatine mass). We can now either tie the artery only, or we can tie the whole heart substance. In either case a ligature of *floss* silk is to be passed round (the artery or the entire heart) and tightly tied and secured. Before proceeding further, we wash out the cavity of the thorax of all blood to keep our bath-water clean, then we lift the animal into the bath and there let it remain ten minutes or so to get well warmed. It is a good plan to slit open the entire abdomen in the *middle* line, so as to allow the warm water to freely get around the abdominal contents: the mass thus gets into every organ and into every part of an organ evenly.

We now connect the pressure bottle with the manometer and with the Higginson's syringe, as shown in the figure, also with the mass bottle. The tube of the mass bottle which is to convey the mass away from the bottle is now clamped, as shown at C Fig. 2, and must never for an instant be allowed to get out of the warm water into the cold air.

Having our small basin full of water, we now squeeze the Higginson's syringe, watching the manometer, to raise the mercury half-an-inch. This done, we remove the clamp from the efflux tube, and the red fluid, after driving out a few air bubbles, begins to flow out, we, at once, make the connection, and all quicksands are passed if we have tied in our nozzles properly into the artery and the connecting part, and fastened in our stoppers thoroughly into our Woulff's bottles.

Our task is easy now: all we do is to seize the head of the animal, which should be to our left, with our left hand to watch the pale gums, tongue, and eyelids become suffused with a pale blush which gradually deepens, whilst we gently squeeze and relax the barrel of the syringe and glance at the mercury from time to time. When the mercury has risen four, or at most five inches, the whole animal will be completely injected: the visible mucous membranes and bowels will be *dark* red and much swollen.

We now remove the animal, and place it in ice cold water or under a common water tap for an hour or two, and divide it into parts as required.

This method of applying pressure is wonderfully delicate; thus, whilst we can raise the mercury in the manometer almost imperceptibly, one entire squeeze of the barrel raises the mercury one inch.

HOW TO PRESERVE BOTANICAL SPECIMENS.

Botanical specimens can, with great advantage, be cut fresh as they are gathered. This is not always convenient, however, so that we require a preserving medium. Preserving them is simplicity itself, as all we require is to place them in equal parts of methylated spirit and water, where they may remain for any length of time.

ON ANIMAL AND VEGETABLE SECTION CUTTING.

No part of histological work has undergone such a rapid change as the so-called "section cutting." Almost within the last twelve months changes have taken place, which have rendered the art almost ridiculously simple, if one may be allowed the expression. By means of Cathcart's microtome and a common plane iron, the most unskilful and unpractical can cut sections of animal and vegetable tissues, of exquisite thinness, at the rate of sixty or seventy per minute. We shall mention other microtomes, and we shall have to point out the short-comings of each. Indeed, the one we have mentioned is, like all things human or of human origin, not absolutely perfect.

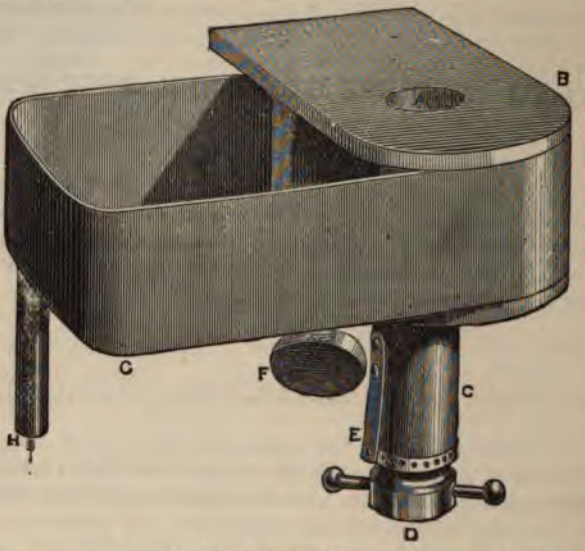
Most tissues, especially animal tissues, are now cut by the freezing process: cutting by hand and razor is practically obsolete: cutting by imbedding and the well-microtome of Stirling is still in vogue, and, in some cases, such as in wood sections, will probably remain so. Speaking generally, however, animal tissues are cut with a freezing microtome.

We now pass on to notice the four principle freezing microtomes, and in doing so, it will be best to take them in their chronological order.

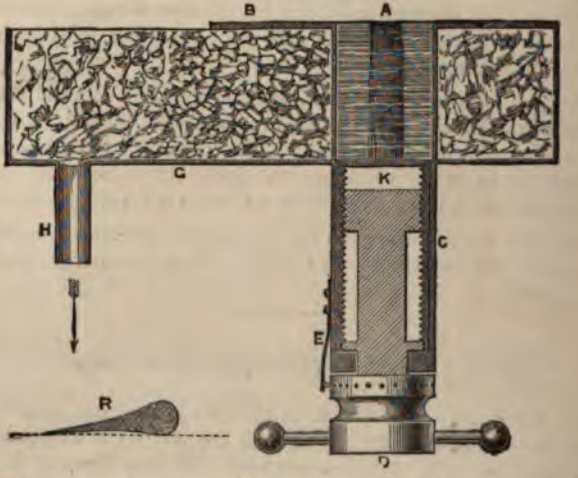
Professor Rutherford's Microtome.

This is Stirling's original well and screw-microtome, plus an ice box arrangement. It is used by filling the ice box with well-powdered and mixed ice and bay salt, equal quantities. Whilst freezing is going forward the well may be covered with felt. One or several tissues can be cut at once, either with a Rutherford's knife, or, what is now found to be far better, a plane iron. By a plane iron, we mean the iron taken out of a carpenter's smoothing plane. The iron must be $2\frac{3}{4}$ inches broad: they

cost one shilling and sixpence each, and are either used with or without a covering of wood. When double-faced with wood, the handling of them is more pleasant, but it does not add to efficiency. The iron must be



sharpened on a very smooth hone, and then well stropped. In sharpening, care must be taken to rub the *entire length* of the cutting edge



evenly. This is best done by taking care to rub the edge on the stone in such a way, that *every* portion of its length is upon the stone at the *same* time. In stropping, it will be found most convenient to rest the plane iron against a table edge, and push or pull the strop over it.

The advantage of the Rutherford freezing microtome is that it can be also used as an ordinary Stirling's microtome for imbedding in carrot or wax. Its disadvantage is the same as that of ice-freezing machines of whatever description: that it is cold, disagreeable work filling in the ice and salt, and the former is not always to be obtained, and when once charged, cutting must follow, or the ice melts and gets spent.

Mr. C. Baker, 244, High Holborn, W.C., sells an excellent Rutherford's freezing microtome, which, of course, must have its cutting face covered with plate glass.

Williams' Freezing Microtome.

This is made by Messrs. Swift & Son, 81, Tottenham Court Road, London, W.C., who also make a registered knife carrier, which carries the blade of a razor: the whole goes under the name of "Swift's Knife." Until the plane iron was thought of, Swift's Knife had the field all to itself most undoubtedly, and there will still be those who will prefer it to the plane iron.

This freezing microtome must be used either with Swift's knife or some contrivance of a similar nature. This combination has this great advan-

tage: that in winter, when ice is plentiful, the machine is charged, and freezes strongly for an hour or more. For class work this is excellent: the attendant charges the machine just before the class meets, and

the demonstrator cuts a hundred sections of any prepared tissue in a few minutes. If he wishes to cut another specimen, he clears away the first, and clears out the grooves on the top of the section carrier with the back of a knife: then he places his specimen upon the machine, and paints it round with gum solution, and the whole freezes fit for cutting in one minute or so. With very little practice, it is quite easy to cut fifty excellent sections of each of fifteen or twenty specimens with one charge of ice and salt.

To manage Swift's knife, we proceed thus:—First take the blade away, and lay it flat upon a smooth hone well oiled, and rub it first on one side, then the other, until *the entire edge* on either side is ground by the hone at once. The writer rubbed over twenty hours before this was accomplished with the razor blade of his Swift's knife. Both sides of the blade are ground quite flat, to begin with, not hollowed; but cutlers either cannot or will not grind a blade with its back and its edge *perfectly* parallel, so that this has to be done by one's self. Having ground the knife blade, we fix it in the handle and strop it. We then take off the handle and fix the blade in the carrier in such a way that the edge must be perfectly level with the top of the microtome, and on a much lower level than the back. This position of the knife in the frame is to the last degree essential, and we effect it thus:—First place the knife-blade in the frame, and lower the screws which the edge of the blade rests upon, so that the edge is lower than the back a good deal. After making it firm, we complete the levelling by freezing a piece of tissue, and taking away a thick section, cutting away from us, with the knife in the frame just as we have put it roughly. We now place each end of the blade alternately against the *same* part or bit of the frozen tissue, and raise or lower either end by screwing either of the two screws nearest to us. All is now complete, and we cut away section after section by lowering the knife edge for each fresh cut by giving the screw furthest away from us a part of a revolution.

To Fill an Ice Microtome.

Get a piece, or pieces, of ice the size of two closed fists, and the same bulk of either common table salt or, what is better, bay salt. Powder both, and thoroughly mix them. The ice is most readily reduced to fine powder by being surrounded with flannel and bruised with a broad-faced hammer, or a carpenter's mallet. Fill in the mixed ice and salt and press it well down, but take care that the trough of the microtome is not so full that the top touches the ice and salt. The top is to be placed on the microtome and screwed fast. In winter the machine, after being charged, is ready for freezing in five minutes or so; in summer it may be ten minutes or more. Freezing proceeds, as we have before said, for an hour or two. The waste pipe at the bottom of the microtome is to be connected by caoutchouc tubing, with a basin to receive the melted ice.

The disadvantage of Williams' microtome is, that, like all ice microtomes, it is disagreeable to charge even when ice is plentiful, and cutting must take place at the time the machine is charged; that is to say, if the operator is called away, as in medical practice often happens, his ice charge melts and has to be renewed.

Fearnley's Ether Freezing Microtome.

Dr. Groves suggested the conversion of Williams' ice freezing microtome into an ether freezer. This is now called the Grove-Williams microtome, and is made by Messrs. Swift and Son, and used with Swift's knife.

For those who are not made ill by the inhalation of atmospheric air charged with ether, the best and simplest ether freezer for use with Swift's knife is Fearnley's ether freezing microtome. This is simply the top

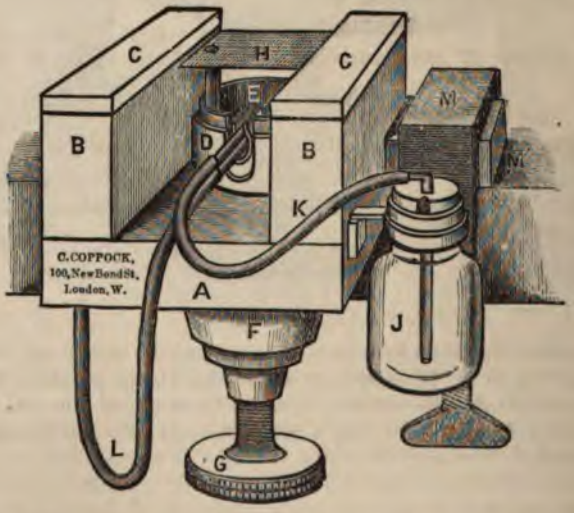
of the Grove-Williams' microtome, supported by three or four legs. The ether nozzle is immediately under the frame of the glass plate, and the bottle of ether stands beside the machine on the table as shewn in wood-cut. Fearnley's microtome is also made by Messrs. Swift and Son.

Cathcart's Microtome.

This is an ether-freezing microtome for use, either with a stout razor, or with a plane iron. Cathcart's invention, used with a plane iron, for

cheapness, simplicity, and efficacy, cannot be approached by any ether or ice microtome we know of. Its cost is only some fifteen or sixteen shillings, and is, therefore, well within a student's means. The plane iron costs one shilling and sixpence; and Messrs. Richardson, chemists, Leicester, sell methylated ether at one shilling and fourpence per pound, which answers admirably. With the expenditure of two drams of ether, the operator can cut sixty or seventy sections in almost as many seconds, every section being of exquisite thinness.

A little gum solution is first placed on the plate, and almost frozen: then the tissue to be cut is placed upon it, and surrounded with gum solution, and the whole frozen. The tissue is *elevated to the knife* by a very small fraction of a revolution of the screw with the left hand, whilst the right drives the plane iron. The iron must be held with the edge *far* below the level of the rest of the iron, and the screw movement and the push of the iron movement, must take place alternately. When a mass of sections has accumulated on the iron, it must be floated off into a saucer of water. After freezing and cutting about half-a-dozen specimens, the operator will startle himself with the rapidity of his own movements. To anyone used to working with mechanics' tools, the *feel* of the iron travelling over the glass and cutting through the tissue as through cheese, is extremely pleasant. We could like to see the screw of this microtome made much thicker, and the threads finer, and the head of the screw half as wide again in diameter. With these slight improvements, in our opinion, the microtome would be absolutely perfect.



Cathcart's Microtome, like all ether freezers, depends mostly upon the nozzles being good. Mr. Coppock, of 100, New Bond Street, who sells Cathcart's Microtome, is very particular that the nozzles he sends out are

examined and approved by a competent judge—the test of a good spray nozzle with this Microtome is the time taken from the spray commencing to act to the first appearance of ice. With this Microtome and good nozzles the time is from five to ten seconds. Bad nozzles take fifteen or twenty seconds, and the spray is coarse and a larger quantity of ether is used. Two drams of ether for *each* specimen in ordinary temperatures is ample. When once the specimen is frozen through, the whole is cut into sections without a single jet of spray further.

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Gum and Syrup Preserving Fluid.

The very greatest objection to the use of freezing Microtomes was the impossibility of taking spirit hardened material and cutting it without an eighteen or twenty-four hours' preparation. Up to a few months' ago anyone wishing to cut by freezing had to take his specimens out of spirit, cut them of convenient size, and soak them in water for twelve or more hours, to get rid of the spirit, then place them in gum solution some hours further. This was a great draw-back, and rendered it a necessity that the operator must think over what he wished to cut, and prepare it through *twenty-four hours previously!*

All this is changed. Specimens are now kept the year round, if the operator chooses, in gum and syrup, having a little carbolic acid in it, and he freezes and cuts any tissue so placed *at any moment* he likes.

To make the gum and syrup medium :—

Take of Gum mucilage[1] (B.P.), 5 parts.
[2]Syrup, 3 parts.

Add five grains of pure carbolic acid to each ounce of the above medium.

Tissues may remain in this any length of time. For brain, spinal cord, retina and all tissues liable to come in pieces, put four parts of syrup to five of gum.

The operator will do well to make the gum mucilage and syrup separately, and keep them so till wanted.

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To cut Tissues, soaked in Gum and Syrup Medium.

Take a piece of tissue, not more than an eighth of an inch thick, and press it gently between a soft cloth to remove all the gum and syrup from *the outside* of the tissue. Set the spray going, and paint on the freezing plate a little gum mucilage : then put the tissue upon this, and surround it with gum mucilage, with a camel hair brush.

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[1]Gum mucilage B.P. is made by placing 4 ounces of picked gum acacia in 6 ounces of distilled water, and stirring occasionally until the gum is dissolved. This is to be strained through muslin.

[2]Syrup is made by dissolving 1 pound of loaf sugar in 1 pint of distilled water, and boiling.

NOTE.—The reader will see that the tissue is saturated with gum and syrup, but surrounded when being frozen by gum mucilage only. This combination prevents the sections curling up, on the one hand, or splintering from being too hard frozen on the other. Should freezing have been carried too far, the operator must wait a few seconds. It ought to cut like cheese.

CUTTING BY IMBEDDING.

Whilst vegetable fungi and all friable vegetable tissue is best cut by the gum and syrup process, stems, petioles, etc., also animal tissues fresh from spirit, are cut by being imbedded in carrot and placed in the microtome of Stirling, or Rutherford.

The operator requires a nest of cork borers, one of these must cut a plug of carrot *exactly* to fit the well of the microtome. This plug must be an inch in depth. He is now to split this in two equal halves by a *vertical* cut. He now takes a cork borer the size of the tissue he wishes to imbed, and gouges the interior of the halved plug, so that on being placed together the two halves gently but firmly grasp the tissue. Now he sinks the plug, grasping the tissue, into the well of the microtome, and does so in such a way that the slit of the plug will be in a line from left to right of him as he sits cutting. By doing this his razor has not to cut through the entire plug every time, but only the half nearest to him.

The knife must have its edge and back *strictly parallel*, or it must be ground so after purchase. It must also be hollow ground on both sides, or at least on the side which rests on the cutting surface of the microtome—*which must be of glass* in all cases. Mr. C. Baker, 244, High Holborn, W.C., sells the knife we allude to.

He must have a soup plate filled with methylated spirit and water, equal quantities, also a saucer of the same, on the table in front of him. *Every time* he cuts a section he must dip his knife in the soup plate and carry a pool of fluid and place it on the surface of the microtome, so as to keep it flooded. Each section is carried into the saucer by the knife. Of course he must have a pail in front of him on the floor to catch waste spirit and water.

Carrots are to be obtained the year round, almost. Those fond of messes can imbed in wax, or in cacao butter, etc. We only mention this way of embedding to condemn it, as quite unnecessary and dirty in every way.

All things which spoil by imbedding can be *frozen* and cut, whether they be animal or vegetable.

STAINS AND STAINING.

It is now usual to bring into prominence the various elementary tissues of which a section may be composed by staining them with one, two, or more stains. Up to the present time, no stain has been found to equal logwood for certainty and permanency of results, and beauty of colour, which, besides being beautiful, is also not too tiring for the eye. We go further, and say that the more a histologist departs from a use of logwood and adopts other stains, the more unsatisfactory will be his total results. If ten men were each to make for himself a histological cabinet, the work of each being equal in other ways, the one who would produce the best cabinet would be found to have used logwood and picro-carminate of ammonia for the great majority of his slides, using other stains which have been found to suit special cases, such as aniline-blue-black for nerve centres, methyl-aniline for amyloid or waxy degenerations in pathological histology in a few cases only. He would further have been found to have used benzole-balsam as his mounting medium in the case of his logwood stains, and glycerine jelly for mounting his picro-carmine slides. Such a cabinet would last a thousand years, and be as perfect the last day as on the first. On the other hand, the worst cabinet especially after, say about 10 years, would be found to have been composed of a *few* logwood slides, mounted in dammar varnish, and the great majority stained with all sorts of aniline and other fancy dyes, and mounted in glycerine. The dammar preparations would be found to be little better than fine grey dust, and the fancy dyes to be conspicuous by their absence. So far as can be judged by our present data a preparation stained with logwood, and mounted in balsam is unchangeable; so is a preparation stained with picro-carminate of ammonia, and mounted in good glycerine jelly.

With these preliminary remarks we now proceed to give formulæ for those stains, *and those only*, which have been found really good in every way. As staining is yet in its infancy, we daily read of a fresh stain, and a new method of staining. We need scarcely to draw the attention of our readers to the present mania for "rushing into print," and the numerous worthless, not to say senseless, communications to our various journals on the subject of dyes for histological work. We advise the histologist to ask himself this question:—Is it my object to make for myself a complete, educative, histological cabinet, or to investigate the subject of stains, and therefore to experiment with the various stains? The operator should settle this question once for all, and *before* he commences his work.

AQUEOUS LOGWOOD STAIN.—Take 60 grms. of *dried extract of haematoxylin*, 180 grms. of powdered alum and rub them thoroughly together in a mortar, adding *slowly* 300 c.c. of distilled water: mix carefully, then filter. To the filtrate add 20 c.c. of absolute alcohol and preserve in a stoppered bottle. The solution should be kept in a cool place for at least a fortnight before using. The older this solution, the more excellent it becomes. HARRIS AND POWER.

ALCOHOLIC LOGWOOD STAIN.—Take a sufficient quantity of 70 per cent. alcohol, absolute alcohol, calcic chloride, powdered alum, and haematoxylin and mix thus: Make three saturated solutions,

a. Of calcic chloride in 70 per cent. alcohol.
b. Of powdered alum in 70 per cent. alcohol.
c. Of hæmatoxylin in absolute alcohol.

Mix one part of *a* in eight parts of *b*, and to this add *c drop by drop*, until a deep purple colour results. Use only a small quantity of absolute alcohol in making *c*, as the hæmatoxylin is very soluble. The colour deepens and improves by keeping. KLEINENBERG.

CARMINE STAIN.—Place a dram of the best carmine in a mortar, and pour over it two drams of strong solution of ammonia and let it stand for twenty-four hours. Take four ounces of a saturated solution of borax in distilled water, and rub down the carmine and ammonia with it; then filter and keep in a stoppered bottle.

NOTE.—The operator takes his choice of logwood and carmine as a stain as they are used in exactly the same cases as a rule. Most people prefer the logwood as being less tiring to the eye.

PICRO-CARMINATE OF AMMONIA.—This stain, commonly called picro-carmine, was introduced by Ranvier, and is a double stain. Make it by dissolving 2 grms. of the picro-carmine in crystals in 100 c.c. of distilled water, in other words a 2 per cent. solution, and filtering. The picro-carmine is sold by Mr. Martindale, of 10, New Cavendish Street, Portland Place, W., also by Messrs. Hopkin and Williams, Cross Street, Hatton Garden.

SULPH-INDIGOTATE OF SODA.—Make a saturated solution of sulph-indigotate of soda in distilled water. Keep this in a stoppered bottle. The sulph-indigotate of soda is sold by Messrs. Hopkin and Williams.

ANILINE BLUE-BLACK.—Dissolve 1 decigram in 4 c.c. of distilled water. To this add 100 c.c. of rectified spirit, and preserve in a stoppered bottle. This stains rapidly, and gives a pleasant slate-grey colour, and is of great value for the nervous system, especially the nerve centres. STIRLING. Sections are to be mounted in benzole balsam.

IODINE GREEN.—Keep a saturated solution of this in a two-ounce bottle, using distilled water. When required, make a one per cent solution,

also with distilled water, and filter. Iodine Green is a multiple stain, really, as it gives different tints of the same colour.

The above stains are the usual every-day stains used for normal histological tissues. *Gold*, *Silver*, and *Osmic Acid*, have already been described.

On Staining.

With Logwood.—Take a few minims of either the aqueous or the alcoholic solution as may be required—usually the former—and place them in a dram of distilled water, then filter into a watch glass. Before placing the sections in it they must first be placed in a 1 p.c. solution of sodium bicarbonate if the tissue has been hardened in any chromium or any acid medium. After this they are to be washed in distilled water of 30° to 40° C. They are now placed in the watch glass of filtered solution of haematoxylin for five minutes, or as long as required. The time is judged by taking out a section now and then, and washing it in distilled water and noticing the depth of its colour. When the colour is deep enough, it should never be very deep, the sections are to be placed in spirit for

Dehydration.—All sections, stained or unstained, to be mounted in balsam, must first be *dehydrated*, *i.e.*, deprived of all their water. To effect this, it is only necessary to place them for 15 minutes in mythelated spirit, or for 5 minutes or less in absolute alcohol. After this, they are ready for

Clearing.—By clearing is meant the rendering of the tissues more *transparent*. To effect this, clove oil and rectified oil of turpentine are used. The former is usually used alone, but it is better to use *both*; the oil of cloves first. Place the sections from the dehydrating spirit into the oil of cloves until they sink, which they will do in a few minutes, more or less. After they have sunk, transfer them to oil of turpentine, which dissolves and disperses the oil of cloves, and (which is very important) clears away any dirt or contamination the sections may have accidentally acquired.

Note.—Another very important use of oil of turpentine thus used is that it enables the operator to mount his specimen in balsam if he so desire immediately: the balsam dispersing, or combining with the oil of turpentine.

Carmine Staining.—Wash the sections in distilled water then in a 1 per cent. solution of sodium bicarbonate and again in distilled water. Now transfer these to the carmine staining fluid, which is placed in a watch glass, from one to five minutes as may be required. When stained sufficiently, transfer them to methylated spirit, and after washing them in this, transfer them to a mixture of

methylated spirit, 5 parts; pure hydrochloric acid, 1 part. This sharpens their colour, and takes out superfluous staining. They must be closely watched whilst in this mixture, or it may take out all the colour. From one to five minutes is the time during which they remain closely watched, as we have said, and removed the instant the process has gone far enough. They are tranferred from this mixture to methylated spirit, in which they may remain till required for mounting.

They are to be cleared and dehydrated exactly as before described, and mounted in benzole balsam.

PICRO-CARMINE STAINING.—Pass the sections through distilled water, 1% sodium bicarbonate, and again through distilled water, as in carmine staining. After this transfer to a 2 per cent. solution of picro-carmine for as long as is required. The time in which sections are to remain in this stain varies from a few minutes to twenty-four hours. They seldom are over-stained by it. They are to be transferred directly to the mounting medium, as will be presently described.

SULPH-INDIGOTATE OF SODA STAINING.—The sections are first stained in carmine as though we were intending to mount them after staining in carmine only. The last process (washing in spirit to get rid of all the acid) must be most thorough, such as allowing them to remain in a large quantity of it for a couple of hours. They are then transferred to the sulph-indigotate spirit. This is made by adding about two drops of the saturated aqueous solution, as described above, to the ounce of methylated spirit, and should be freshly made for each time of using. The sections are to be placed in a good quantity of this (two or three ounces) for about four hours, not less. It is best to place the sections and sulph-indigotate spirit in a wide mouthed stoppered bottle during the time the staining is going on.

ANILINE BLUE-BLACK STAINING.—Wash the sections in distilled water then transfer them to the stain as described above. When pretty deeply stained clear, dehydrate and mount in Benzole Balsam.

IODINE GREEN STAINING.—Take the sections *fresh* from spirit and place them upon the surface of a 1 per cent. aqueous solution of the stain. The sections float upon the stain until the spirit they contain is displaced, and then they sink. When just about to sink, *instantly* remove them to distilled water and wash, then clear, dehydrate and mount in Benzole Balsam.

MOUNTING.

Slides.—It is customary to mount microscopical objects on glass slides or slips three inches by one in England. On the Continent this is not so generally the rule and gives rise to inconvenience when an English microscopist purchases a slide of another dimension, the slide may not fit the cabinet. Of course, the slide ought to be chosen according to the object mounted, but we do well to adhere to the usual size when possible. The quality of the glass should be looked to; a slide should be of thin sound clear glass, and neatly ground on the ends and sides.

Covers.—These are square, round, and oval in shape. When we have our choice, rounds are best, if we wish to finish a slide neatly by running around the cover a little cement. Squares are excellent, especially for mounting in Balsam. If, however, we have an oblong object to mount, an oblong or an oval cover should be chosen. The thickness of the cover is highly important, and ought always to be marked upon the label of the slide, though this precaution is often neglected. In consequence of the rays of light being diffracted by the cover, the thick cover glass ought to be avoided except in special cases. Cover glasses are sold in qualities marked No. 1, No. 2, and No. 3, those of the first number being the thinnest and most expensive. Covers are sold by the ounce, and an ounce of No. 1 covers ought to contain hardly any cover over ·006. An ounce of No. 1 covers ought to have a fair proportion of ·003 inch glasses in it; still more of ·004 than ·005 advancing in proportion from ·003 to ·006. Should an ounce of No. 1 covers contain more than a dozen or at most a score *over* ·006 it ought to be returned. As the objectives now used are immersion, or ought to be, for powers higher than an eighth the thickness of the cover may be of less importance. For instance, Messrs. Powell and Lealand's $\frac{1}{12}$ oil immersion lens, even when worked with its high angled front, works through a cover of ·006 inch most perfectly. Almost anything in histological work can be seen with an objective of a $\frac{1}{6}$ inch power: only exceptionally do we require so high a power as the $\frac{1}{12}$-inch. We ought, however, to use no cover thicker than a ·006 inch, if possible. There are numerous cover-glass measures. Messrs. Ross make an excellent one, but it is rather expensive. As most

amateurs would be provided for amply by a very few ounces of covers, to purchase a measurer out and out seems to all but the wealthy a hardship. With Ross' cover-glass measure we measured an ounce of No. 1 covers, and placed each in its proper place (in this case, pill boxes marked on the lid according to size of contents), in less than half an hour.

Cleaning Covers and Slips.—This process differs according to the dirt or matter to be removed. In covers and slides that have not been used, it is best to place them in strong sulphuric acid for an hour or two: then transfer them to methylated spirit for an hour or two. We now take the covers out, one by one, and rub them with a clean old silk handkerchief very carefully. The slips are rough dried, on any clean rag, then rubbed clean with chamois leather. When slides and covers have been used for balsam, or have had zinc cement run on them, or any hard substance usually employed in mounting, they ought to be placed in a strong solution of caustic soda for a few hours, then rough dried with a clean rag and polished—the covers with a clean piece of old silk, the slides with chamois leather.

Labels.—We should like to see more information on labels than is usually the case. A model label should be divided into two parts, one for each end of the slide. The one ought to have upon it the name of the object, its origin, and its mode of preparation; thus, suppose it be a section of lens, we should have "Lens T.S. from Rabbit, prepared in Pot. Bichrom and Spirit." The words "from" and "prep. in" should be in print; the former occupying a place a little below the middle of the left side of the label; the latter a little below this again. The other half of the label ought to tell with what the object is stained; what it is mounted in; the thickness of the cover; and the date of mounting. The printing would be "Stained" "Mtd. in" "Cover ·00" "Date." The words occupying the extreme left of the label in the order from above downwards we have given, taking care to leave a small space after the two ciphers for the last of the three numerals to be inserted in writing, thus, "Cover ·006," the 6 would have to be written by the one who mounted the specimen. We have only to add that if the labels were tastefully margined, and *evenly* printed on sheets of paper—of various tints to suit the tastes of the various purchasers—each sheet containing one of the halves of the labels only, they would be a boon to histologists. Most labels as now sold have nothing upon them but a bleared, thick, unsightly margin, and they are printed upon the sheet of paper as evenly as if they had rained upon the sheet, so that we require a special kind of scissors, *not yet invented*, to prevent cutting every second label in halves as we labour to cut evenly around *the* label we are endeavouring to isolate.

Transference of Sections.—Thick sections, such as are used for opaque objects, can be handled anyhow; but sections only the $\frac{1}{600}$ inch thick,

and still thinner, have to be transferred from place to place with great tact and care, or they are torn and disfigured. Sections are transferred by floating; by camel hair brushes; by lifters, &c. Many histologists recommend the first as the only method of dealing with the more delicate sections. By practice, however, the two latter methods may be safely used. Excellent section lifters are sold by Mr. Stanley, Optician, London Bridge, and others. They should be kept of two sizes for large and small sections. Further, one of each size should have holes drilled through it to freely allow fluid to pass through. This is advantageous, as it allows the lifter to retain the section with certainty, whilst a section in water or spirit will float away from a lifter not so perforated in consequence of the thin layer of water which remains between the surface of the lifter and the section, carrying the section away. The lifters not perforated are used to transfer a section from the clearing oil to the slide. In this case a thin layer of oil must remain between lifter and section or the latter will *not* float off, but will have to be dragged off, and will very likely be torn. We advise amateurs to transfer sections by means of brushes and lifters, but until they have acquired the art of doing so without injuring the section, they will do well to avail themselves of the floating method in the case of their delicate and valuable sections.

1. *The Floating Method.*—When sections are transferred *en masse* from one vessel to another, they may be boldly poured in like water alone, using a wide funnel, and ignoring the presence of the sections: from a saucer into a wide-mouthed bottle, for instance. When a section is to be landed upon the exact centre of a slide: when it is a very thin tender section, and when it requires skill to prevent creases, we effect this by holding our slide in our left hand and a camel hair brush in the other. We then dip the slide into the fluid in which the section is floating, and gently bring its exact centre under the section, and place the brush upon the edge of the section nearest to our left hand: we now withdraw the whole. If we find the section in creases, and not lying flat on the slide, it is easy, whilst we hold it with the brush on its left border, to float it out, and spread it out evenly before altogether withdrawing it.

2. *Transferring with brushes.* Small camel hair brushes with few but long hairs are used. The brush is merely dipped under the section and raised. The section then sits like a saddle upon the brush and can be transferred to another fluid by the most unpractised without injury. If, however, the section is to be spread out on a dry slide the matter is more difficult. In such a case we allow the most dependent part of the section to come down on the slide first and the slight drag of this part on the slide enables us to get the brush from under the section by revolving the brush on its long axis *away* from that portion of the section which is touching the slide. This manœuvre is a little difficult, but is a most useful method of transferring a section to a slide and at the same time spreading it out flat.

3. *By Section Lifters.*—We insert the lifter beneath the section with our left hand, whilst with our right we hold a mounted needle or a brush, to fix the edges of the section nearest to our left upon the slide and withdraw the whole. If the section be lifted from the clearing oil to the slide the superfluous oil is got rid of by touching a dry clean slide once or twice with the edge of the lifter. If, however, we drain away all the oil, and the section is delicate, or large and delicate, it will adhere to the lifter and be torn in attempting to draw it upon the slide from the lifter, which we do by our needle, or brush. It requires therefore, some experience to stop short of withdrawing too much oil from the lifter and section. It is a fault in the right direction to float any amount of oil upon the slide from the lifter rather than have to drag and tear the section after withdrawing too much oil. To get rid of the oil on the section as it lies on the slide is quite easy. We incline the slide on its end by rearing it against any perpendicular body, and the oil drains away down the slide, whose lowest end should rest on blotting paper. After a little while we bring a piece of blotting paper upon the section, whilst the slide is lying section upwards, and stroke our right hand twice over it *firmly*, exactly as we should do were it wet ink on a sheet of notepaper. The section, however delicate, is safely and thoroughly deprived of its oil by this means, and not injured in the least. The blotting paper may have to be brought down on the section in two fresh places rapidly, one after the other. This leaves the section ready for our benzole balsam.

MOUNTING.

(Continued).

Description of Materials.

Glycerine $C_3 H_5 3 H O$ is the hydrate of the trivalent radical Glyceryl. It is a sweet syrupy liquid, obtained by the decomposition of fats and oils, principally as a by-product in the manufacture of candles and soaps. The fatty acids are used to make candles and soaps, when combined with soda or potash. Pure glycerine is colourless and odourless, freely miscible with water and alcohol in all proportions; but with oils it only emulsifies, and does not perfectly blend. It is a solvent of many alkaloids and their salts, as well as resins. The purist is prepared by distillation: although not volatile without decomposition, yet it passes over undecomposed in the vapour of water, and may be concentrated by careful evaporation. This mode of preparing it, was patented by Price's Candle Company, but now much distilled glycerine is imported from Germany. Glycerines of inferior quality have a disagreeable smell, and are sometimes coloured. Good glycerine should not be coloured after being subjected for two hours to the action of an added solution of the nitrate of silver.

Glycerine Jelly.

Take of

Gelatine	300	grains
Distilled water ...	6	ounces
Glycerine	6	ounces
Rect. Spirit	6	drams
White of Egg ...	6	drams
Salicylic Acid ...	12	grains

Let the Gelatine soak thoroughly in the water, then dissolve in a water-bath: add the spirit, and mix well. When cool, but still fluid,

add the white of egg, mix and heat to boiling point to completely coagulate the albumen : add the glycerine with the salicylic acid in it by the aid of heat : mix well and filter, while still hot, through paper previously moistened with distilled water ; the whole should be kept in a hot chamber whilst filtering. (Martindale.)

Canada Balsam.—This is the oleo-resin obtained from *Abies Balsamea* and *Pinus Canadensis* and is mostly imported from Quebec. The Balsam is obtained from the tree by puncturing the blisters, or vesicles, which form under the bark of the trunk or its branches, and collecting their fluid-contents in a bottle. It is at first opaque, but gradually clarifies and becomes transparent. It consists of 24 per cent. of volatile oil, 60 per cent. of resins soluble in boiling alcohol, and of 16 per cent. of resins soluble in Ether, but insoluble in alcohol. As it dries slowly, it is, for histological purposes, generally evaporated until brittle, and dissolved either in Chloroform or Benzole. These solutions dry rapidly, and are perfectly transparent.

In order to prepare Canada Balsam for histological use it should be *gently* heated over a sand-bath until it becomes quite hard and brittle. If the heat be too great it burns the Balsam, and renders it brown, and therefore spoils it. When quite dry and brittle it is to be broken up into small pieces and dissolved in Benzole and filtered for use.

Asphalt.—This is also called mineral pitch. It is a hard, brittle, black or brownish black resinous mineral found in many parts of the world. It breaks with a resinous fracture and melts at about 100° C: easily takes fire : is soluble in chloroform, ether, benzole, volatile and fixed oils ; but only partially so in alcohol.

White Zinc Cement.—This is made as follows :—

Take of	Benzole	... 8 parts
	Gum Dammar ...	... 8 parts
	Oxide of Zinc ...	... 1 part.

Mix the gum dammar and the benzole, filter through cotton wadding, after which mix in the oxide of zinc in a mortar and again filter through the wadding. (Woodhead.)

To mount in Glycerine.—Place the slip on a turn table, and run upon it a ring of asphalt. The ring should be the size of the cover, which, of course, should be a round cover. When the asphalt is half dry, but still sticky, place the section on the slip, and put over it a drop of glycerine ; then lower the cover over it in the usual way, and gently press the edge of the cover all round, so as to make it adhere to the asphalt. Should it not do so at any point, gently warm the asphalt by holding the slip over the flame of a spirit lamp, then press

the cover home. The slide can now be dipped in water, and any glycerine that has overflowed or been pressed out, washed off with a camel-hair brush. After drying it can be ringed with white zinc cement.

To Mount in Glycerine Jelly.—Warm the jelly in a water bath. This is most readily effected by placing the bottle containing the jelly in hot water—an old tinned meat can filled with water, and placed over a spirit lamp or Bunsen's burner is a good and cheap water bath. The jelly must only just be melted, any extra heat being prejudicial. The jelly is landed on the slide with a small hollow glass rod or cylinder closed with the index finger of the right hand. The section should first be placed in the centre of the slip, and well surrounded by the medium—water that has been boiled, or distilled water—from which it was taken: the jelly is run upon it in great abundance, and the cover lowered upon it.

To Mount in Canada Balsam.—By Balsam we mean Benzole Balsam. This is effected by either of two processes :—the rapid, and the slow, or drying, process. The former has to be resorted to in most cases, but where the operator has time, the latter way is by far the best, as the slides can be placed in spirit, and the superfluous balsam cleared away immediately the cover is turned over.

The quick or instantaneous method of mounting in balsam is simply to place a drop of it on the slip or cover, then to lower the cover upon the slip.

The slow or exposure method consists of allowing twelve or eighteen hours to elapse before placing the cover on the slip—the drop of Benzole Balsam being exposed to the air during this time. It is as follows :—Supposing numerous sections are being mounted, a clean slip is breathed upon, and three clean covers are stuck upon it, each cover being placed on the slip the *instant* after a deep expiration of breath has bedewed the slip. The extremely minute layer of moisture suffices to make the cover sit fast on the slip. A section is placed on each cover, then a large drop of Balsam is placed on each section, and the slip with its three covers, &c., is placed somewhere out of the way of dust. At the end of twelve or eighteen hours the covers are turned over on to well cleaned slides, the latter, *not the covers*,[1] being

[1]This slow method of mounting in Balsam was discovered by the Editor many years ago, and he has freely taught it to many pupils. The pages of a contemporary recently contained a grotesquely worded description of the process, which otherwise was moderately correct; the writer, however, directed his audience to warm the *cover (!!)* in the flame of a spirit lamp—the cover with the section upon it ! !

The writer, in describing the process, declared it was a process *he had discovered after much labour and time spent in experimenting.* The *discovery* was made by peeping over Messrs. Cole and Son's shoulders, like a second Paul Pry, during the few months this person worked in Messrs. Cole and Son's Laboratory.

warmed by being held in the flame of a spirit lamp a few seconds, which causes the balsam to run freely as the cover is lowered upon the warmed slip. A very minute drop of fresh benzole balsam may be placed upon the extreme edge of the cover, next to the condensed balsam, if thought desirable. This minute drop is made to touch the warmed slip first, and mingles with and sets going, so to speak, the condensed balsam.

Ringing slides with Cement.—Slides which contain sections mounted in Glycerine Jelly must have their superfluous jelly scraped off with a knife and then be washed in water. Slides with Balsam mounts must be placed in a plate containing methylated spirit and then rubbed with a cloth dipped in the spirit until all superfluous balsam is removed.

When dry and clean the slide is placed upon a turntable and centred accurately. This is done by watching the rim of the cover as the table rotates; it is the cover that must be centred, and, therefore, the cover *rim* must not deviate in any direction as the table rotates.

White zinc cement is put on; then, after this is dry, a ring of asphalt is run upon the zinc ring to give the whole slide a smart appearance.

The brushes used must be rinsed in benzole and dried in the air.

Care of the Hands.—If the hands get stained with magenta or other dye which cannot be washed off in the ordinary manner, they may be thoroughly cleaned by washing in hot water, containing "washing" soda and a table-spoonful or two of chloride of lime.

MOUNTING.

(Continued).

Full and special directions have been given under the heading "METHODS OF PREPARATION" for the mounting and general treatment of the various animal and vegetable organs and tissues, which have been described in the essays forming Volumes 1 and 2 of the "STUDIES IN MICROSCOPICAL SCIENCE," for the preparation of "hard" sections of bone, teeth, &c., and of Petrological specimens. It is now necessary to give practical instructions in the mounting of various preparations.

THE PREPARATION OF DIATOMACEÆ.

Diatoms may be said to be absolutely ubiquitous; recent and fossil, living or dead, they are readily obtainable in vast numbers, and of infinite variety. In nearly all waters, fresh or salt, still or running, in marshy districts, on the surface of the ocean, or attached to algæ and shells, they abound in a living and growing state, whilst their siliceous valves, after the death of the cell-contents, are to be found, in a more or less perfect condition, as deposits at the bottom of rivers and ponds and on the sea floor. From the stomachs of holothurians, ascidians, molluscs, and of various shell-fish, exquisite varieties have been obtained in profusion. As fossils they form beds of enormous extent, intermixed with the siliceous casts of polycystina, radiolarians, &c., and the calcareous shells of foraminifera, whilst fossil deposits purely diatomaceous and yielding the most beautiful, rare and varied forms have been discovered in all parts of our globe. In order to clean diatoms and render them fit for mounting, as either "selected" or "strewed" slides, considerable experience and great care are necessary. The sub-siliceous varieties especially require very delicate treatment, whilst many of the fossil and recent forms, which are intensely siliceous, must receive altogether "heroic" and drastic measures in order to ensure perfect cleansing of the surfaces of the valves. There have been nearly as many nostrums propounded and published for the cleaning and mounting of Diatoms as there are genera and species of the Diatomaceæ themselves. Years of experience and constant experiments have led us to the conclusion that the simplest and safest modes of treatment are the best. For living Diatoms—and especially for the sub-siliceous forms (*e.g.*, Pleurosigma) the following method will give perfect results. Remove all dirt, débris, and salt, by repeated washings; thoroughly shaking up the gathering each time and allowing the Diatoms to settle before pouring off the water; in this way all soluble impurities can be removed. When the water remains clear, after shaking, pour it

off, leaving the Diatoms as nearly as possible dry; cover them to about one inch in depth with absolute alcohol, or the strongest ordinary alcohol, this will gradually dissolve and extract all the endochrome from the valves; change the alcohol daily until it ceases to be tinged with green. Wash off all trace of alcohol, pour off all water, place the Diatoms in a platinum capsule and heat them to a dull red over a "Bunsen" burner; this will separate the frustules into single valves, and put the finishing stroke to the cleaning of the Diatoms, which may be now bottled up in distilled water for future mounting.

To Clean Diatoms growing upon algæ or shells.—Place the algæ or shell débris in a *large* basin (in order to allow space for effervescence), cover them with water, add hydrochloric acid, and stir until violent effervescence results; add acid, little by little, until effervescence ceases—thoroughly stirring from time to time—strain through net of sufficiently fine texture to admit of the Diatoms passing, but to retain the débris. When the forms shall have thoroughly settled down, pour off all water, and place the strained deposit in a large test-tube. Boil in pure hydrochloric acid for twenty minutes; add pure nitric acid, and boil again for twenty minutes. Whilst boiling add crystals of chlorate of potash until complete bleaching results; remove all trace of acids and alkali by repeated washings, and examine the forms; if perfectly clean bottle them up for mounting; if, as is sometimes the case, there has been animal matter present, which has not been entirely removed, boil in pure sulphuric acid for a few minutes, and wash away all trace of the acid before bottling the Diatoms in distilled water.

To Clean Fossil Diatomaceous Deposits.—Some fossil deposits are of such extreme hardness that it is necessary to resort to caustic potash for their disintegration; it is, however, better, whensoever possible, to dispense with this powerful alkali since, even in the most careful and practised hands, more or less abrasion of the surface of the valves will be found to result from its use. When a fossil deposit will not yield to milder treatment, it must be broken into small pieces, placed in a test tube, just covered with the solution of caustic-potash, and boiled for *half-a-minute (only)* over the flame of a "Bunsen" burner; pour off into a glass beaker nearly full of water all the deposit which has been disintegrated by the potash and repeat the process until all the lumps are broken up. Then, when all the forms have settled to the bottom of the water in the beaker, pour off the water, remove the Diatoms into a large test tube, fill the test tube nearly full of water, add a small quantity of bicarbonate of soda, and let the Diatoms gently "simmer" in this weak solution for two hours. Wash away all soda, and boil the Diatoms for a few minutes in pure nitric acid. Wash away all trace of acid, and bottle up the Diatoms in distilled water.

Fossil deposits which can be disintegrated without caustic potash should be boiled in a test-tube of large capacity, in a moderately strong solution of bi-carbonate of soda—the disintegrated portions being, from time to time, poured off into a beaker and the boiling in bi-carbonate of

soda repeated until all the deposit has broken up; when the alkaline solution should be washed away and the Diatoms boiled, for a short time in nitric acid—all traces of which are to be removed by repeated washings and the forms bottled up in distilled water for future use.

To mount Diatoms "dry."—All "dry" mounts of Diatoms, whether "strewed" or "selected," are liable to destruction or deterioration from an accumulation of moisture upon the under side of the cover, which moisture, sooner or later, and in defiance of all precautions, always makes its appearance. "Dry mounts" are therefore always, more or less, unsatisfactory and unreliable and to be avoided as much as possible. The best method of mounting Diatoms "dry"—whether for "test" or as "arranged" slides—is to make a cell of the *best* asphalt, of the necessary thickness by adding coat upon coat of the asphalt, *not* by making the cell of sufficient depth at one operation. Spread the Diatoms upon the cover, if necessary "burn" them upon the cover, *i.e.*, place the cover upon a piece of thick platinum foil and raise it, slowly and carefully, to a dull red heat over the flame of a "Bunsen" burner; thoroughly heat the slip with the asphalt cell upon it; whilst it is *hot* (and therefore *certainly* free from all damp or moisture) place the, *equally hot*, cover carefully upon the cell, pressing down the cover and making sure that it adheres thoroughly and evenly to the cell—run a ring of asphalt round the edge of the cover; when this is hard, ring the cell with two coats of white zinc cement, letting the first coat dry thoroughly before applying the second.

To mount Diatoms in Canada Balsam, &c.—Canada Balsam has been, hitherto, universally employed as the best medium in which to mount Diatoms. It has stood the test of time, and proved fairly permanent and reliable hardened Balsam re-dissolved in Benzole or Chloroform is, however, by no means so satisfactory in its results as ordinary Balsam, since it does not admit of being submitted to the application of direct heat, after the slide is mounted; any attempt so to harden the Balsam, resulting in the production of air-bubbles, which carry away the forms in the ebullition caused in the endeavour to drive out the air, and without heat, applied directly, the Balsam never "sets" hard. Diatoms should therefore be mounted in chemically pure, filtered Balsam, diluted to the consistency of treacle with pure turpentine. The Diatoms in "strewed" slides should be allowed to fall upon the cover from a "dipping-tube" held at a height of three or four inches above it—the sudden fall of the drop causing the Diatoms to spread evenly upon the surface of the cover[1]. The drop upon the cover should then be dried, very slowly, by means of a "Bunsen" burner placed underneath the brass table upon which the cover (lying upon a 3 × 1 slip) has been placed. When it is desired to mount many slides of the same gathering of Diatoms the requisite number of covers should be cleaned, and three of them laid

[1]The even spreading of the Diatoms on the cover will be further ensured by breathing upon it before allowing the drop to fall, whilst in selecting Diatomes if the cover upon which they are to be placed is breathed upon the forms will adhere to it.

upon a slip, previously breathed upon to cause the covers to rest firmly upon it, and the slips laid upon the brass table; a drop of water containing the Diatoms should then be allowed to fall upon each cover, as previously recommended, and the covers can all be dried together, a sufficient quantity of the turpentine-balsam should then be put upon the covers, which should be placed in a cabinet with trays, or under a glass shade, so that all dust may be excluded, for 24 hours. By this means the perfect permeation of the forms by the Balsam, and the consequent removal of all *air* are secured *before the final completion of the mount*, an advantage so obvious that further comment is unnecessary. The covers should then be replaced upon the brass table and subjected to gentle heat from a "Bunsen" burner until the Balsam is hard, the cover should then be taken up by means of forceps, and a slip being made hot the cover should be placed precisely in the centre of the slip, which should then be held over the "Bunsen" burner until the Balsam runs to a neatly bevilled edge round the cover. No air-bubbles will trouble the mounter if this process is carefully practised. This is the so-called "exposure-process," which was invented by the Editor in 1870, and the success of which induced him to devise the necessary modifications upon it and to construct the necessary appliances in order to adopt it to the mounting of histological and other preparations.[1] Slides of "selected" Diatoms should be mounted in the same manner, the forms being picked up by means of a carefully chosen hair from the dried hide of a cow. The soft and flexible hairs taken from the *under side of the neck* being those most suitable for the purpose. These hairs are to be affixed to the cedar sticks used with "camel-hair pencils" by means of sealing-wax dissolved in alcohol, and a number should be prepared having the hairs of different lengths in order to obtain varieties of strength and elasticity. Some hairs should be mounted of considerable length, as the mounter of Diatoms and other such minute specimens must learn to put them back symmetrically into their required positions *under the microscope*, and *in the Balsam* when they float away, a little practice will soon lead to the acquirement of this *absolutely necessary* skill, and it will be found not difficult to heat the *hardened* Balsam (many times if necessary), and whilst it is hot to, very rapidly, replace a form in its desired position, whilst with a fine needle all hairs and other imperfections can be removed and absolute cleanliness ensured.

[1] In order to ensure the placing the cover exactly in the centre of the slip, either of the three following devices will be found convenient:—

Firstly. Upon a small white tile draw, in china painting colours (which should be burnt in) a 3 × 1 space (representing a slip), with its precise centre strongly marked. This tile will be found of great use in suddenly cooling Balsam, when that may be desirable.

Secondly. Cover a 3 × 1 slip with white paper, rule lines faintly from each top corner to its *opposite* bottom corner, the *intersection* of these lines will give the exact centre of the slip, which can be marked by a dark spot.

Thirdly. With a writing-diamond make, upon a slip, placed on a turn-table, concentric circles, ¼in., ⅜in., ½in., and ⅝in. in diameter; each sized cover can then be placed precisely in the centre, if the slip on which the cover is to be placed is laid upon that upon which the circles have been described, the circles, of course, being clearly visible through the super-imposed slip.

For a considerable time experiments have been in progress in the hope of discovering a medium, in which to mount Diatoms, which should be more satisfactory, in respect of its refractive index, than Canada Balsam and equally reliable and permanent; many such media have been tried—phosphorus, monobromide of naphthaline, and others; but it is unnecessary to enter into details concerning them since the desired results have recently been obtained by Dr. Van Heurck, who has succeeded in providing a *perfect* substitute for Balsam in gum-styrax, which yields the best possible results. It will probably be found that Diatoms mounted in gum-styrax are less liable to accidents than Balsam "mounts," as the latter becomes resinous in time, and the covers are liable to "spring," the result of which is the appearance of prismatic colours in the Balsam, which are not only a great eyesore, but sadly deteriorate the slide. Gum-Styrax may be considered absolutely permanent and unalterable. Purified Styrax contains a granular substance, which must be removed by dissolving it in chloroform and filtering the solution. The solution thus obtained is used in the same manner as Canada Balsam. Styrax solution is even easier to work with than Balsam, and air-bubbles are not produced in it by the application of heat. The crude Styrax as purchased, should be exposed in a thin layer to the sun for several weeks. In this way much of its yellow colour is discharged, the water it contains evaporates, and it becomes hard. It may then be dissolved in chloroform, as already described. Instead of chloroform, benzine, or a mixture of benzine and absolute alcohol, may be employed in making the solution, whilst a solution in ether will prove valuable when rapid "setting" of the medium is desired.

The Mounting the Diatomaceæ.

For a considerable time experiments have been in progress in the hope of discovering a medium, in which to mount Diatoms, which should be more satisfactory, in respect of its refractive index, than Canada Balsam, and equally reliable and permanent; many such media have been tried—phosphorus, monobromide of naphthaline, and others; but it is unnecessary to enter into details concerning them, since the desired effects have recently been obtained by Dr. Van Heurck, who has succeeded in providing an admirable substitute for Balsam in gum-styrax, which yields the best possible results. It will probably be found that Diatoms mounted in gum-styrax are less liable to accidents than Balsam "mounts," as the latter becomes resinous in time, and the covers are liable to "spring," the result of which is the appearance of prismatic colours in the Balsam, which are not only a great eyesore, but sadly deteriorate the slide. Gum-Styrax may be considered absolutely permanent and unalterable. Purified Styrax contains a granular substance, which must be removed by dissolving it in chloroform and filtering the solution. The solution thus obtained is used in the same manner as Canada Balsam. Styrax solution is even easier to work with than Balsam, and air-bubbles are not produced in it by the application of heat. The crude Styrax, as purchased, should be exposed in a thin layer to the sun for several weeks. In this way much of its yellow colour is discharged, the water it contains evaporates, and it becomes hard. It may then be dissolved in chloroform, as already described. Instead of chloroform, benzine, or a mixture of benzine and absolute alcohol may be employed in making the solution, whilst a solution in ether will prove valuable when rapid "setting" of the medium is desired.

Cleaning and Mounting Polycystina.

The siliceous shells of these lovely organisms, the beauty of which is only equalled by their variety, are found in profusion, and intermixed with Diatomaceous valves, often of extreme beauty and rarity, in the deposits (or so-called "earths") of Barbados, the Bermudas and the Nicobar Islands, whilst an entirely new and magnificent deposit was discovered by the late Captain Perry, of Liverpool, at Jérémie, Haiti, which contains not only a large number of novel species of Polycystina, but many entirely new varieties of Diatoms.

The Polycystinous "earth" should be broken into small pieces, about the size of a nut, and boiled for half-an-hour to an hour, in a strong solution of common "washing soda;" the disintegrated portions being poured off into a large vessel containing clean water, from time to time, and the boiling in soda repeated, as also the pouring off, until the whole mass is perfectly broken up. When the disintegrated matter in the large vessel shall have thoroughly settled down it should be subjected to several washings, in order to remove the soda, the material being allowed to settle thoroughly after each washing. It should then be removed to a beaker or wide-mouthed bottle which should be filled up with water, and after being thoroughly stirred or shaken up, the material should

be allowed to settle for *thirty seconds* only, and the supernatant fluid and its floating particles poured off into a large vessel; this process should be repeated 3 or 4 times, and will give the heaviest density of sand and Polycystina. Repeat this process with the matter in the large vessel, allowing it to settle for *two-and-a-half to three minutes*, and the density containing the small Polycystina will be obtained. Subject the remaining matter to like treatment, allowing it to settle in six inches of water for 20 minutes, and the density consisting of the débris of Polycystina and of Diatoms will result. Now boil each separate sediment in nitric acid for fifteen to twenty minutes, remove all trace of acid by repeated washings, and finally boil each density in a weak solution of Bi-carbonate of Soda in a test-tube of large capacity for an hour; this will remove all flocculent matter, and, after repeated washings, perfectly clean Polycystina will be obtained. The heaviest density consisting of sand and the largest Polycystina should be placed in a test-tube with about three inches of water and subjected to rotatory motion, this will cause the Polycystinous shells to rise above, and to free themselves from, the sand and they can be poured off into a wide-mouthed bottle or beaker, the sand being left at the bottom of the test-tube. This latter process should be carefully conducted and repeated several times in order that no large and perfect shells may be left amongst the sand. Each density should then be bottled in distilled water. Polycystina may be mounted in Balsam—in the same manner as Diatoms—or "dry." Beautiful slides can be produced by calcining the shells upon a piece of thick platinum foil, or in a small platinum capsule, and mounting them "opaque." The neatest and best method of preparing such slides is to make a disc half an inch in diameter in the centre of a slip, allowing it to harden for some days. A half-inch cover is then to be cleaned and a sufficient quantity of Balsam, thinned with turpentine, put upon it; the Polycystina are then to be placed in the Balsam in sufficient quantity to form a surface over the cover when evenly spread upon it; the cover with the Polycystina is then to be put aside (as recommended with Diatoms) for 12 or 24 hours in order that the Balsam may thoroughly permeate the forms and all air escape; the Balsam is then to be hardened by gentle heat, as already described, and the cover is to be laid upon a slip, with the Balsam upwards, and held over the flame of a Bunsen burner until the Balsam is liquified; the Polycystina are then to be evenly spread upon the cover by means of a needle; when the Balsam is cold a small drop of Benzole-Balsam is to be placed upon its surface and a clean half-inch cover to be warmed and carefully lowered upon the Balsam. The Polycystina will thus be mounted between two covers; now, turn the "mount" over, so that the under surface of the lower cover shall be upwards, and coat this with Asphalt, put the mount aside for a day or two, in order to allow the asphalt to become thoroughly hard, then put upon the asphalt disc on the slip a small drop of (cold) "French Liquid Glue,"[1] place upon this the asphalted

[1]This is to be obtained from any chemist, and is a most admirable, reliable, and cleanly cement.

under surface of the "mount," and when the glue is quite dry finish off the slide with either asphalt or white zinc cement.

The Preparation and Mounting of Insects.

There are several processes for preparing and mounting insects, each of which possesses special advantages, in respect of the results and appearances it may be desired to obtain. Whenever it is possible, and except for special purposes, and in dissections Insects should be mounted "without pressure," since the flattening them out under pressure results in distortions, displacement of the parts and organs, and unnatural, and therefore, false appearances. It is, however, impossible to mount all insects, or their parts, without pressure, and the following process will give good results :—

Place the insect in pure Liq. Potass : mixed with ¼th ammonia fort. The insect must not be allowed to remain too long in this solution, and must be tested from time to time by placing it in water and pressing the thorax. When the thorax is soft and the legs flaccid immerse the insect in water for from 15 to 24 hours, then soak it in glacial acetic acid and glycerine (half and half) for some hours.[1] Again, immerse it in water for from 12 to 24 hours ; it is now ready to be laid out upon the slip ; having done this, preserving the natural position of the parts as closely as possible, place a cover over the insect and tie lightly round it with soft cotton, stand the slip on end to drain, plunge the slip into a turpentine bath and leave it until all moisture has been driven out and the insect is thoroughly permeated by the turpentine, drain off the turpentine, apply blotting paper to remove any excess of turpentine from under the cover, and run in "Benzole-Balsam" by capillary attraction. The cover should never be lifted or allowed to shift its position after the insect has been laid out.

When it is desired to preserve the brilliant or delicate colours of an insect it should be placed, immediately after being killed by means of Chloroform, in Liq. Potass, *without any mixture of Ammonia*, for from two or three days, then soaked in water for 24 hours, then placed in water, with 10 drops of muriatic acid to each ounce of water, for 24 hours, it should then be laid out and immersed in turpentine and treated as already described.

In order to mount insects, or their parts, "without pressure," it is generally necessary to adopt only the following simple process :—Soak the insect for two days in equal parts of ordinary alcohol and water, after which transfer it to absolute alcohol for two days, immerse it in turpentine until it is not only completely permeated therewith and all air removed but let it remain until it is sufficiently bleached or decolorised—in other words, rendered transparent—and in order to ensure this result it should be placed in a strong light. Select a cell, of the requisite depth, of pure tin or vulcanite, which affix to the centre of a slip with the French

[1]Insects removed from Liq. Potass, after being soaked in water for 24 hours, should be kept in strong, or glacial, acetic acid and glycerine (half and half) until required for mounting.

Liquid Glue; when this is dry, having cleaned the interior of the cell, place within it the insect, and fill the cell with fairly thick Balsam until it presents a slightly convex surface above the cell, lay it aside, as previously recommended, under cover for twelve to twenty-four hours so that all air may escape, put a minute drop of fresh Balsam upon the surface of the Balsam which fills the cell, and carefully place a cover, slightly warmed, upon it, close the cell by gentle and equable pressure, at once remove, by means of a soft brush and benzole, all Balsam which has exuded under the pressure, let the mount harden for a day or two, and then apply white zinc, cement or asphalte.

Cells are to be obtained of various sizes, having pure tin caps to fit exactly over them, after the cover is applied, and these not only impart great strength and security to the cell, but ensure the neatest possible finish to the mount. Mr. F. Enock, whose exquisite entomological preparations are altogether unrivalled, is to be credited with this admirable device, as well as with various other improvements in the special branch of the art, to which he has devoted himself with such singular success.

The Preparation of Vegetable Sections.

Whensoever possible stems, leaves, roots, petioles and wood should be cut fresh and sectionised as soon as may be—all such specimens should be kept in a mixture of equal volumes of alcohol, glycerine, and water. Sections also may be stored in this mixture. Nearly all vegetable sections require bleaching before being stained; very delicate tissues may be bleached by means of alcohol—hard and deeply-coloured stems and woods must be bleached in a liquid thus prepared :—

To one pint of water add 2oz. of fresh chloride of lime; shake this up thoroughly two or three times, and allow it to stand until the lime shall have settled. Make a saturated solution of common "washing soda." Pour off the supernatant fluid from the chloride of lime, and, by degrees, add to it the soda solution, until all precipitation ceases. Filter the solution, and keep it in a stoppered bottle, in the dark. No fixed time can be given for the bleaching process, the colour and density of tissues being so variable. Experience, however, will be rapidly gained, and over-bleaching easily avoided. The sections being bleached, must now be washed in distilled water, several times changed, and allowed to remain for twenty-four hours in the final water, to which add 8 or 10 drops of nitric acid to each half-pint. Transfer the sections to alcohol, for an hour before staining them. To bring out details of structure and to display cell walls, &c., there is no better stain than Logwood, and for singly stained vegetable sections it is to be recommended in preference to all other staining fluids. The following process will give admirable results :—

1st. Remove the section from alcohol to water for a few minutes.
2nd. To 3 per cent. alum solution for 10 minutes.
3rd. Stain in aqueous logwood stain. (See page xlii.)
4th. Place in alum water to remove stain from surfaces.
5th. Wash thoroughly in water.

6th. Place in alcohol for two or more hours. Float the section lightly on the surface of oil of cloves: when it sinks it is ready to be mounted in Balsam.

To Double Stain Vegetable Sections.

1st. Place the section in an alcoholic solution of Iodine Green (3 grains to the 1oz. of alcohol) for one or two hours.

2nd. Soak it in alcohol for ten minutes.

3rd. Remove it to water for one minute.

4th. Immerse it for two hours in Carmine fluid, made as follows:—Carmine, 15 grains; Ammonia, 15 grains; Water, 2oz. Dissolve the carmine in the ammonia by means of gentle heat, add the water, and filter.

5th. Wash thoroughly in water.

6th. Place in alcohol for ten minutes.

7th. Float upon oil of cloves and mount as previously described.

Mr. Gilburt, whose vegetable preparations are most successful, recommends the following double-staining fluid, in which the sections are immersed, and which produces perfect differentiation. Dissolve of Magenta Crystals $\frac{1}{4}$ grain in 1 ounce of alcohol, and of Nicholson's soluble blue $\frac{1}{8}$ grain in 1 ounce of alcohol; add to this 4 drops of nitric acid. Filter both solutions.

To 2 parts of the blue add 7 parts of the magenta solution. Immerse the section for from 1 to 2 minutes, remove it to absolute alcohol, thence to Benzole (to fix the magenta); thence to oil of cloves, and mount in Balsam.

Picro Carmine, Double Staining.—Picro Carmine as a selective double stain cannot be surpassed. The process is as follows: Take of carmine 2 grains, liquor ammonia $\frac{1}{2}$ drachm, distilled water 1 ounce; dissolve the carmine in the ammonia by means of gentle heat, add the water. Dissolve 8 grains of picric acid in 1 ounce of alcohol, also by means of gentle heat, and mix the two solutions.

Place the sections in alcohol for one hour—immerse them in the *recently filtered* staining solution for from half an hour to three hours—*i.e.*, until they are sufficiently stained,—wash them in alcohol, immerse them in an alcoholic solution of Picrate of ammonia for one hour, and for a second hour in a like solution, in other words, change the solution once during the two hours. Place them in alcohol for a few minutes, and in oil of cloves as already described, and mount in Balsam.

ON MICROSCOPICAL DRAWING AND PAINTING.

A great teacher has said "Drawing should be considered not an accomplishment, but a necessity. Learning to draw is learning the grammar of a language. Anybody can learn the grammar, but whether you have *any thing to say* is another matter." To the naturalist this accomplishment is of great importance; accurate illustration adds to the value of written description. At every point the microscopist is sensible of its deeper significance. Such a control quickens the perception, excites exact observation, and creates an interest beyond research, admiration, or curiosity. The compactness of the vision, presented by the microscope, so rivets the attention, that changes, disclosures, development of activities, in organisms often lost and swept away, after cursory examination, rouse in a zealous observer an impatient desire to possesss some power, beyond words, to place on paper a memorandum or record, however rough, of things rarely discovered under the same conditions. This ability is a result of practice. There is no royal road but that traversed by enthusiasm and earnestness. Sketches from the hands of a dexterous microscopist, marking first impressions, are often more valuable and superior than the formal work of the mere draughtsman, who may not even *know* the significance of the subject, especially when the result is a replication of drawings made by the actual observer. He necessarily falls into one or other of the errors; he mends and improves, or obscures material points by drifting into formal monotony; a microscopical draughtsman must essentially be a microscopist, and work *direct from actual observation*, completely understanding the matter before him.

There are three well defined characteristics of microscopical representations, drawings of tissues, or minute organisms, requiring for elucidation high powers, delicate conditions of light, conducted under careful observation and technical skill, satisfying the highest biological research, in its progress demanding rigorous precision; then, rapid sketching, catching features, graphic memoranda; without hesitation, or the assistance of the camera-lucida jotting down, and washing in, with tints, unexpected appearances, this readiness should be cultivated by those desirous of adding record to observation; many most important phases in the sequence of activities have been seen and passed over, when a few rough lines would have induced and helped further research, but beyond this tentative work, and the stern formality of scientific requirement, is a finished "picture;" at this crucial point the capability of the microscopist

and artist blend, involving knowledge of the subject, the arrangement of optical apparatus, judgment, and study in the methods of procedure. A drawing may be true in its scientific aspect, and possess artistic features of decided interest—the one may incorporate the other. The illustrations of Mr. Gosse's books are instances of this peculiar quality. "Still Life" has arrested the attention of artists, of all time, from Missal Illuminators to Royal Academicians, such results have no scientific import, but like all art products, awaken gratification in appreciating the power applied in producing their essence, and, without degrading the legitimate functions of the microscope, it is possible to extract from its revelations, models of exceptional charm and excellence, associated, moreover, with scientific value. Although the bias of an expert microscopist and practised artist may not often touch the same mind, it is certain that when a keen perception is directed to complications of beauty, with rare conditions of light, and effulgence of colour, the instrument becomes the very touchstone of artistic feeling, and, beyond mere beauty (which, in visible nature, is inexhaustible) there are revelations of structural form, quaint elegancies, mysterious changes of tissues, and embryological developments, under radiances, hidden, not only from ordinary familiarity, but even from the cognisance of many who have not had the opportunity of exhausting the resources of a fine instrument, with all its accessories. It may be urged that few have the ability to approach art of this description, but the power of drawing, quickly develops itself, especially when stimulated by special and eager interest, concentrated on special objects; no one led by inclination to contemplate what may be seen under such circumstances, can be destitute of an appreciation of art in its most exalted sense. The education of the eye (the basis of æsthetic culture) as exercised by the fascination and mental excitement of microscopical research, progresses in a degree hardly yet understood, or appreciated; every student has within his mastery this power; the manipulation of the instrument, the means of display, the use of materials, are matters of expertness, and are extensively self-taught, possibly some instruction may do more than groping alone, but in the end experience is the best master. It is proposed in this essay to detail such an actual experience in words, directed more to mental judgment than technical education.

A microscopical drawing may be absolutely true, and an artistic grace secured, by preserving line for line what is actually presented, assuming the preparation to be fairly perfect, in other words, not drifting into a stilted diagrammatic style, or wandering from close observation, because the subject *appears* to have a certain regularity; no two cells, vessels, or fibres are absolutely alike; to give "life" to a picture, every part of the structure should be a portrait, the pencil deviating from accuracy melts into falsity and confusion, *uniformity is fatal*, and obscures important differentiation of parts; again, in order to delineate what is expected, or wished to be seen, aiming at "correction," is to be avoided; it is better to draw imperfections, if they be present, an overlapping or torn structure often reveals an important fact, so patent is this, that a "fabricated"

drawing may be detected in a moment, especially of Diatomaceous or Infusorial forms—a broken fragment, a solitary individual is the clue to a perfect whole, or group; such built up arrangements have no charm beyond technicality. A good representation possesses a mingled quality of accuracy and imperfection, a paradox, which stamps its value! Suppose a preparation of vertical section of human scalp of rare excellence, double stained, disclosing beauty in many perfect hairs traceable in their course direct from the base of the bulb, embedded in the follicle, and emerging from the cuticle above. In cutting a section of such delicacy it would be impossible to avoid slicing through a hair or two diagonally, thus leaving the tops of some, the ends of others; this result or defect, is a feature of significant interest from an art point, faithfully copied it gives life and character. In a diagram, the imperfection, by comparison with perfect hairs, might be remedied, the mutilated parts "restored;" but such an interference destroys at once the graphic quality of the picture, adding nothing to its scientific interest. Absolute accuracy in depicting what is presented may, however, in some cases, be qualified, and truth evolved by a knowledge of the structure as it *should* appear, particularly in cellular tissues, in close contact. In such cases the artist ought to be cognisant of elementary forms, as arranged under contiguous pressures, and the position of spherical, oblong, or cubical elastic cells, as affected by juxtaposition in, over, or under spreading layers. Coupled with the perspective of such conditions, this facilitates progress. In opaque subjects, under binocular vision, where the rotundity of a reticulated surface fades in dimensions, and shadow, in different lines, this abstract knowledge is important, and should be acquired, as many objects could not be effectively represented without its study—always keeping to general appearances; it is an ability which removes difficulty in unravelling the disposition of parts, especially under high powers; when sections are cut either too thin, slightly oblique, or disrupted by the knife, the mechanical interferences of parts when understood, may be restored. The functions of an artist, cognisant of a condition of antecedents may be fairly exercised in the progress of a drawing, but it must never trench upon absolute truth and discrimination in treatment—a drawing may be ruined in a moment by a false line involving impossibility of structure; to a critical eye, this is fatal. In fine work, dealing with malpositions, shrinkage of tissues, disseverances and pseudo-appearances—inevitable even in the finest preparations—the utmost judgment is required.

The effect of a microscopical drawing is enhanced by its inclusion in a circle—surrounded by a black margin—forming a square. The size of the circle is important—it may be too large, or too small; experiment proves that a space three inches and three-quarters in diameter approximates nearest to the impression made on the mind, of a "field" as seen with a B eye-piece, this circle may encompass magnifications under any power. A metal plate four inches and three-quarters square, with an opening of the dimensions given, should be procured, this ascertained gauge will soon prove a necessity—placed on a drawing block, a pencil

swept round the circle and outside the square gives the interior for the drawing and the lines for backing with Indian ink—these discs should be prepared before the work is commenced, and the importance of this arrangement will be shown hereafter.

The help derived from the camera-lucida is strictly limited, it cannot be employed beyond a certain point, no elaboration can be effected by its prolonged use, it should be discarded the moment its legitimate purpose of marking points and positions is achieved; those experienced in its employment always feel a sensible physical relief, and "breathe again" when it is set aside to settle down to the earnest work of direct vision; its application to the instrument is sufficiently familiar, the microscope rendered monocular by withdrawing the prism, the camera-lucida is slipped over the A eye-piece—which should always be used (higher eye-pieces expand the field beyond the fair range of the instrument), clamping the object, the microscope is depressed into a *true* horizontal position (if not, a distorted picture would be the result) and the lights adjusted, the distance from the object to the eye-piece should be nearly equivalent (if anything a little more), to the reach from the eye-piece to the paper. With a microscope standing ten or twelve inches high (a Ross No. 1) this condition would reveal the phantom of the object, outside or about filling a circle of the dimensions given on the drawing block, if any difference of over, or within, lapping appear, it may be remedied by raising or otherwise adjusting either the paper or the microscope so as to obtain a perfect coincidence of the vision and the circle, the importance of a measured disc is now manifest, proportion is affected by distance from the eye-piece, and with this gauge and a stage micrometer, a drawing may be kept to measurable bounds; difficulties have existed as to amplifications expanded by the camera-lucida, absolute accuracy may be ascertained by the use of micrometers as far as the eye-piece is concerned, but the *known diameter* of a circle on which the image is projected, is an easy factor in such calculations, beyond this, the circle is mechanically useful, if the block should slip when using the camera-lucida, there is an ascertained line for re-adjustment. The light on the paper should be in excess of that from the object; speed and precision are essential, quickly make recognised points and lines, never attempt to draw detail, nothing fatigues the eye or distracts the mind more than the prolonged employment of the camera-lucida. No advance can be made by its continued use, any attempt at elaborate work ends in confusion. Cultivate the "knack" of seeing at the same time, and in the same position, the reflections and the image of the tracing point (the hardest pencil, sharply cut); it is not necessary to strain the sight to keep the *entire* field always in view, there is a condition of steady gazing, the eye not too close to the prism, when PARTS of the object can be taken separately, but this is a result of the facile use of the instrument. The neutral tint glass or any form of reflector, giving only one, and, consequently, a reversed image, is useless, for afterwards continuing a drawing from direct vision, but with any description of camera-lucida, the pencil, once placed on the paper, should not, if possible, be lifted

until all determining lines are fixed; the eye (unsteady at the best) and the pencil point must be in unison. Keeping the pencil on the paper preserves "the place."

In arranging any object for drawing, it should be sufficiently magnified to shew everything bearing upon its elucidation, and, as a rule, an isolated subject, a complete form, ought to occupy, as nearly as possible, the entire field. Some specimens necessarily overflow the circle—surfaces of injected preparations, botanical sections exhibiting features requiring the highest magnification, consistent with the preservation of a focal plane, which, obviously, cannot be fairly disclosed (except at a loss of important detail), are beyond the scope of the circle of popular survey. It must then be abandoned, the drawing spread out, and made in sections by shifting, and combining visions. Using the camera-lucida any part may be carefully drawn, making two or three (if angular, the better) prominent points, corresponding with similar appearances in the subject. These marks or tri-angulations (as near the margin of the field as possible), must be remembered, the position of the object is then moved by stage adjustments, and another part of the field arranged, the included marked points are coincided, by shifting *the block or paper*, and further outlines expanded, in this way the camera-lucida may be used under high powers with four or even six combinations of vision—and the parts, with care, will "read into" each other, and result in a drawing of considerable dimensions, perfectly mapped, and true in contour, it may then be continued part by part.

After faint outlines and points of certainty are securely indicated, the microscope is placed in position, and with B eye-pieces drawing from direct observation commenced; prolonged work is facilitated by removing the caps of the eye-pieces; when attention is continually diverted from the instrument to the pencil the fatigue is lessened by keeping the eyes some distance from the glasses, cultivating a faculty of losing the recognition of the *entire* objects, only directing the alertness of vision to the particular part under consideration, in fact it is not necessary, nor, is it prudent to strain the sight to keep the full blazon of the field under observation, and this rule may, with advantage, be applied to the general use of the microscope. At this point, steady work commences, faint camera indications are studied, lines corrected and strengthened, either with pencil, or better, a fine sable brush or pen, carrying a mere tint of Indian ink or "Payne's Grey." Extreme care is necessary; no mistake of line can be permitted; paper, intended for such drawing, and delicate after colouring, does not permit erasure, or the contact of any rubbing out substance; and consistent with the subject, too much fine *line* cannot be put into the work; no attempt at shading, either with pen or pencil, must be attempted. The lines being perfected, and the subject, as it were, "modelled," the painting may now be cautiously commenced. The absorbent quality of paper (well known to those accustomed to water-colour sketching), interferes with, and sometimes helps, artistic

results; without entering into the rationale, but bearing upon the point, it may be mentioned that no wash or even line should be superimposed or carried over another until the first be *perfectly* dry; stippling should show a granulated appearance, lost when touches are allowed to run into and become absorbed by each other.

Illumination, and its diversities, for art work, are of as much importance as the amplification. Power and light should be adapted to each other, and to the character of the subject, its mode of preparation, and what it is expected to reveal. The light, whether from gas (argand burner), or oil, should be capable (in the case of gas, by means of flexible tubing), of being placed in every possible position, from the surface of the table, to, or even above the level of the stage. Ordinary transparent objects, under low powers, are sufficiently shown with transmitted light from the mirror, modified through a diaphragm of waxed tissue paper, ordinary preparations of insects cannot be better displayed; the best reflected light is from the side speculum, collected from a flame through an intervening plano-convex lens, *on a separate stand*. In all observations, even the simplest, accuracy of *light-focus* (often neglected) is important. For powers beyond the half-inch, transmitted light is aided and improved by the purity and control afforded by the achromatic condenser, an instrument in the hands of novices, not always well managed, or sufficiently appreciated; focussing on the same plane as the object, the source of light; it is capable of regulating intensity, purity, and deviation of rays—by apertures and stops, with which it is supplied, and thus the most varied combinations may be secured. Its use should be thoroughly mastered; as it produces the most beautiful, instructive, and even amusing effects. For instance, with a half-inch objective and full aperture, carefully focus, on a ground glass slip, the flame of the lamp, now interpose a dark stop, which should occupy, in the centre, about one-third of the field; removing the slip, replace it with a group of (say) volvox-globator; the plants will be seen rolling from the outer ring of pure transmitted light, into the central black disc, where they appear like emeralds; free-swimming rotifers will pass backward and forward, from the outer ring of parallel rays, into the eclipse of the dark stop, where they become by oblique radiations, self-luminous; no finer example, as showing in one field, at the same moment, two extremes of illumination, could be placed before the microscopical artist, or an ordinary observer to prove what may be effected by an adept in the use of this beautiful instrument. With low powers striking presentations of artistic illumination are under easy control; in particular, the use of the paraboloid combined with light from above. An experienced microscopist is familiar with all these methods—but the artist, alert and eager for experimental conditions, often hits upon effects not generally applied, possibly sacrificing scientific truth to æsthetic desire; a result of positions, and foci of illuminators—their accurate or eccentric adjustment, cutting off central or peripheral rays, dispersing or half obscuring light by intervening transparencies. The importance of such combinations is

paramount in the examination of semi-opaque objects immersed in a thick bed of medium, without pressure. These preparations are in parts dense, even solid, combined with tissues of extreme delicacy and transparency; nothing being crushed, they shew the impossibility of revealing the correlation, or association of parts, by mere transmitted light—or in any way, except (may it be said) by artistic discernment. The head, and surrounding parts of an insect, prepared in this way, with pure light from beneath and above, discloses a combination of form, and colour, of surpassing beauty, the blaze from the speculum sweeps over the opaque parts with reflections revealing the most exquisite tints, while the paraboloid shows, in actual perspective, the parts beneath in all their natural colour, and bathed in refulgence. An opaque polypodom, touched by such reflections, while the extended polyps are illuminated from below, is another instance, amongst many, of beauty, exalted by light.

For purely opaque objects, the only good light is from the speculum, by no other means can the finest effects of colour and shadow be obtained. It should be fitted to the *stand* of the instrument, not to the stage, nor should it slip on the front of the objective. On the stand it can be moved without disturbing the object or the focus. The old fashioned Lïeburkuhn cannot be used; it requires an object to be prepared in a particular way, and, as an illuminator is palpably defective, the light completely surrounds and enwraps the object; brilliancy is present, but no contrasts. In using reflectors, the lamp should be placed close to the level of the stage, within easy reach of condenser and speculum.

In painting purely opaque objects under top light the treatment of background deserves attention, eggs of insects or parasites, are generally attached to fragments of wood, leaves, cuticles, hairs or feathers—it enhances the effect, and beauty of a representation—if such details are carefully painted, and the rest of the field delicately stippled up with Indian ink, to the edge of the circle. This applies to many subjects—threads of algæ, or vegetable stems supporting such objects, as fixed rotifera, polyzoa, etc., introduced into a drawing, add greatly to the interest and make most attractive pictures. Any prepared mount or specimen should be as perfect as possible, and *considerable experience* is necessary in order to decide, what is fairly *good* as a preparation, and *worth drawing.* Common objects of easy procurement from the woods, the garden, and the stream, are exquisite models for the draughtsman, their excellence, interest, and freshness are necessarily superior to even the admirable results now obtained by professional preparers, aided by mechanical appliances, and rare skill in the use of re-agents and staining fluids.

In illustration of illumination of a *precise* object, as affected not only in appearance, but in colour, reference may be made to the plate, which represents a transverse section of a spine of Echinus under four diverse conditions, the upper division (1) with fairly good transmitted lights, and (2) improved by a condenser, the lower portion (1) under ordinary *reflected* light, and (2) aided by a paraboloid or spot-lens.

It is obvious that objects under polarised light, are practically beyond the power of faithful delineation, in all painting, whether in local tint or shadow—purity of colour and the preservation of brilliancy—is of the first importance; in order to render, beyond a mere semblance, any subject under the polariscope, it would be necessary, if such a power were possible, to dip the pencil into light itself, and an insuperable difficulty exists in the permanent preservation of the adjustments necessary for future work, the slightest touch, or alteration of any part of the instruments; and even an obscure change, beyond all control, in the *quality* of the source of light alters the entire gamut and consonance of colour, impossible to re-establish, yet, if selenite films be dispensed with, some results may, with care, be recorded; petrological preparations, the dichroism of crystals, sections of shell, bone, scales, horn, and other semi-transparent organic structures, of varying densities, reveal points of interest only seen under such conditions and may be noted; but considering that the most exalted light at the command of the artist, is the white of the paper, (in all cases, to be jealously preserved), and that the polariscope discloses the purest *coloured* lights, associated with complimentary tones of every gradation, it is clear how futile are the resources of the palette to depict the lustres and unisons of tones as revealed by this fascinating instrument.

Structure and its thoughtful exposition is the limit of draughtsmanship, and it is here that the photographic lens as a delineator fails, the superiority of work produced by a hand guided by cultivated observation as compared with a photograph is the operation of a mind capable of expressing combined and superimposed tissues, in having at command a control and adjustment of various planes of surfaces, and without militating against scientific truth, seeking for, and obtaining even picturesque effects, this important power is felt when searching the depths of an opaque injection, or peering into intricacies of tissues. The objective used in microphotography, especially if it be a high power (unlike the *penetrating* quality of an ordinary portrait lens), is strictly limited to *one*, and that a very delicate focal plane requiring a fine and FINAL adjustment, enhanced by the difficulty that the visual and chemical foci of microscopic objectives do not coincide, entailing a manipulation which never touches perfect precision; on the other hand, a draughtsman may arrange a minute and just perspective of parts, absent in a photograph, anticipating the presence of relative parts, and having at command the fine adjustment, he can feel his way, conscious that at the slightest touch a fresh point, perhaps an important revelation, flashes into sight, supplying a link to the better understanding of the whole. A drawing, produced under thoughtful guidance, conveys to an appreciative observer an attrac-

In the establishment of a "cabinet" nothing should be admitted, that is procurable, fresh and living, at each recurring season. A "collection" should be strictly limited to typical subjects difficult to procure and requiring special treatment, involving section cutting, injection, dissection, or methods of preservation and revelation, necessary for future study and reference, this would exclude from a cabinet (instances need not be particularised) much, which even degrades it.

tion totally absent in a photograph ; the latter may possess the important and essential element of proportion and freedom from exaggeration, but exactitude is never absent from a drawing disclosing understanding, and conscientious treatment.

There may seem little or no analogy between landscape and microscopical painting, but the same principles are involved—points of sight, effective light, general entourage—possibly a "preparation," dealing with unusual and unexpected complications of line, embracing physiological difficulties, requiring delicate conditions of luminosity, demands a deeper judgment, for it is often necessary to prepare the mind by careful and prolonged study, before the paper is touched ; especially in considering and anticipating difficulties of representation, and how they may be overcome—delicate structures, under the most careful illumination, often appear as streaks of LIGHT, when a slight touch of the condenser may reveal distinct *lines*. These are points to be studied ; in fact, the subject should be "gone over" and *arranged* in all particulars, so that it may not outstrip the power of the pencil. All materials should be of the finest quality, the paper, hard, thin, smooth, and unglazed—delicate pencil drawings may be made on Bristol board, but such or any glazed or hot-pressed surfaces, are totally unfitted to take colour. Fine drawing paper is preferable, when blocks are used each surface must be examined for imperfections with a hand lens ; a delicate painting may be ruined, at a critical point, by an imbedded hair, an abrasion, or minute speck ; in the manufacture of these blocks, it has been found that in cutting up and folding the paper the *true surface* is not in every piece placed uppermost. For important work it is safer to select sheets strained in the usual way, in a *small-sized* folding drawing frame. Paper improves by age. If of undoubted antiquity, it fetches high prices. It is impossible to render satisfactorily, on a white surface, opaque preparations showing minute injected anastomosing veins, arteries, or glands, the dark interstices separating them, cannot be drawn, or picked out, without sacrificing the regularity or destroying the uniform diameter of the vessels—but such subjects may be effectively painted on a dull black paper, which may be previously pasted on a drawing block under pressure, using opaque or body colour, vermillion, yellow ochre, Antwerp blue, and carmine, combined with and regulated for substance, and tint, with zinc white and gum water. Payne's grey, with zinc white, produces the peculiar shadowy hyaline tone so often seen as a substratum in such preparations where semi-transparent spongy tissues are involved—fine effects of receding distances—in following the depths of structures, may be produced by its use. Numerous brushes of sable are required, the hairs short and coming to a fine point, they should be of the best make, no brush that has touched Indian ink can be used for delicate colour, and those employed for carmine, yellow, and blue, should be *marked* and kept distinct, the same applies to pens, often required, but the pen carrying colours must be used with extreme discretion. If a fine line can be obtained with the sable, it is of higher quality, moreover with the handy pen, the temptation is great to obtain hurried results by strokes and

dots, but for pure black and white memoranda, or representations requiring speed, nothing can equal a fine pen, charged with Indian ink, or neutral tint, remembering never to approximate, or cross a line, until it be properly dry; with this precaution a pen drawing may approach the semblance of an etching. The colours should be dry cakes, the palette preserved as pure as possible; moist pigments, rubbed from pans, become contaminated, and even dry cakes should be kept separated; loose and in contact they chip and soil each other. Quality is all important, use only those which are "transparent." Manuals on painting contain lists of recommended pigments, and their qualities, generally meeting no attention; for the work in question, it may remove difficulties, to remember that important colours are neutral tint, Payne's grey, Antwerp blue, carmine, scarlet lake, yellow ochre, Hooker's greens Nos. 1 and 2, and raw Sienna—colours to avoid, vermillion, cadmium, the umbers, Emerald green, and Vandyke brown. These are densely opaque, and "load" too heavily for delicate work; a good test is to rub a portion of each cake of a well-furnished box on a clean porcelain palette, side by side. When dry, those which appear dull and dusty (however useful they may be in landscape in large thin washes), reject: everything may be accomplished with the remainder. There is no difficulty in conducting a painting by artificial light when conversant with the character and combinations of the *few* colours really required, a precaution, however, is necessary in painting tissues stained artificially with logwood or analine dyes; these colours are very deceptive, and differ in appearance under degrees and qualities of light. Logwood stain (often used) in daylight has a blue tinge, under the lamp it appears as a decided port wine tint, and a difficulty may, (in fact, does) ensue in matching day, and lamplight work. When the entire subject has to be painted in the same tone, cakes of "Mauve" and "analine blue" now to be procured may be used alone, and thus stained tissues can be painted under any conditions of light without leading into error. It need hardly be said that such abnormal colours are to be used exclusively for those special preparations, and should never enter into the composition, *or even touch* a general palette required for *natural* representations. Indian ink must be of superlative quality, the difference in price, although not deadly, is great. A piece should be secured, regardless of cost, and treasured.

With practice in cultivating *accuracy* of touch, certainty of line, and forgetting the existence of rubber and knife-edge, no difficulty may be anticipated in drawing on wood, zinc, or lithographic stone.

Crouch End. E.T.D.

ON PHOTO-MICROGRAPHY.

It is too late in the day to claim any originality for the subject of this chapter, since the application of photography to the delineation of microscopic objects is almost as old as the photographic art itself, extending back even to the days of Daguerrotype. Microscopists of the present generation should think of this, and while paying tribute to the patient perseverance with which their forerunners must have worked under all sorts of disadvantages, should blush that, notwithstanding all the recent advances and all the simplicity of the gelatino-bromide process, so few avail themselves of the facilities it affords for the truthful and beautiful delineation of the objects of their study. The chief reason for this neglect is probably the idea among the uninitiated that photography is a very complicated and difficult art, dependent upon a very uncertain condition in our climate—bright daylight—and that unless a person had the necessary day-time leisure and were expert in ordinary photography it would be useless to attempt this special application of the art. It is to expose the fallacy of all this, and to show that any microscopist armed with a small text book and with a simple apparatus which it is quite within the bounds of possibility for him to make for himself, and having no leisure time but the dark winter evenings, can, after a few weeks' practice, produce pictures of objects in his collection which for absolute fidelity, aye, and for beauty, are incomparably superior to the highest flights of the draughtsman's skill. In taking up the study of photography a beginning must be made somewhere, and the tyro's first efforts may as usefully, and with as great a prospect of ultimate success, be directed to this branch as to any other. It is a matter, not of doubt, but of certainty, that his first attempts will be failures, from under-exposure, over-exposure, forgetting to draw the slide, and therefore no exposure, fog, frills, stains, pinholes, under-development, and other causes, but had he commenced with portraiture or landscapes he would have had the same dismal record of good plates gone wrong, and would have had the additional gratification of many an unproductive tramp. Therefore we would say be not deterred from taking up the work because you are not a photographer. Start operations and become one. It will, of course, be impossible here to give any elementary instruction in photography pure and simple. All that can be done is to describe such apparatus and processes as are special to this particular work. For all that is general a good text book of photography must be consulted[1].

[1]Captain Abney's "Instruction in Photography," Piper and Carter, is one of the best.

The apparatus employed is simple. It consists of a microscope of any ordinary construction, a powerful source of light, and a camera. The mirror of the microscope is discarded except for special purposes, because the loss by reflection is very serious. The microscope (see Fig.) is placed horizontally in a line with the source of light, and with its tube inserted by a light tight joint into the front of the camera, which is supported at such a height that its centre coincides with the optic axis of the microscope. The object is held on the vertical stage by means of spring clips, and the light from the lamp is condensed on it by one or more condensing lenses. There are two chief methods in use. In one the eyepiece of the microscope is removed, and the inverted image is received on the sensitive surface where it is first formed. In the other the eyepiece is retained in its place, and the image first formed in its interior is formed again with additional magnification by the aid of the eye-lens. By the use of the eyepiece the advantage is gained that any ordinary camera may be used, and in consequence of the shortness of the distance between the focussing screen and the microscope, the various adjustments of the latter are accessible, while focussing, without any special arrangements. In order to secure the same amount of magnification or to cover the same sized plate, when the eyepiece is not used, the camera must be of special construction so as to be capable of extension, to two or three times the former distance, and when so extended to—say a yard—the focussing screen can only be reached by rods and bands or intermediate gearing. For facilty, therefore, the eyepiece method is commendable, but the impairment of the image and the loss of light due to the interposition of two additional uncorrrected lenses are so considerable that we would advise the removal of the eyepiece for all but special purposes, such as the covering of a very large plate.

The microscope may be of any ordinary construction that will allow the body to be placed horizontally, and it should have a stop to prevent it going beyond that position. It should be provided with a coarse adjustment, by rack and pinion, and a fine adjustment for slow focussing. Very much of the success of the work depends upon the excellence of the slow motion. It is always a very important part of the microscope, but for photo-micrography it becomes very much more so. It should be free from the slightest lateral motion, lest, in focussing, the image be removed from the centre of the plate, a very small displacement of the object glass being sufficient to effect this, when working with high powers and a long camera. It must work with equal smoothness and sensitiveness, and without loss of time in either direction. If the tube of the microscope can be shortened by unscrewing the part above the fine adjustment so much the better, for when working without the eye-piece, a long tube, especially if it be a narrow one, greatly contracts the field. This is one objection to the Jackson Lister form of stand for photographic work, and the difficulty here can only be got over by selecting an instrument with a wide tube. There is another objection to the retention of a long tube, whether the eyepiece be employed or not. It is certain to give rise to a "flare" of light by reflection from its inner surface, and flare, whether arising from this source

or from the setting of the object glass, or the interior of the camera itself, is absolutely fatal to the production of clean pictures, and results in the diffusion of a uniform light all over the plate, which impairs the purity of the shadows and produces a general fog. One chief seat of this internal inflexion is the fine adjustment tube. No amount of dead blackening, or even lining with black velvet will completely stop it. The only thing to be done is to interpose along the course of the tubes of the microscope, and in the camera, and even the object glass itself if necessary, a series of diaphragms. These may be cut out of visiting cards with gun punches, and glued to narrow rings of cork to give them a grip of the tube. They and the corks must be painted dead black with water colour (lamp black), and their number, position and aperture so adjusted that a line drawn from the centre of the object glass to the edge of the tube when at its shortest shall just graze the edges of all these apertures. Thus arranged they will not contract the field, and will not allow a ray of light to fall on anything but the front faces of the diaphragms themselves, whence it cannot be reflected to the plate. When the eye-piece is used the diaphragms must be differently adjusted, for the tube then practically ends at the front surface of the field glass, and its diameter is practically the clear aperture of that lens. A slight sliding backward or forward of the diaphragms will be sufficient to effect this adjustment. In the camera itself the diaphragms, if required, will take the form of sheets of blackened card with central apertures. The writer, who works with quarter plates ($4\frac{1}{4}$" by $3\frac{1}{4}$,") prefers to make the aperture nearest the microscope small and circular, the next larger and shortly oblong, with very rounded corners, the next more oblong and with less rounded corners, and the last oblong with acute corners and the shape of the plate, but a trifle smaller. On looking through the whole length of over a yard of camera and tubes when so arranged, and with a blaze of light streaming in from the condensers, not a single stray beam can be seen, and nothing is visible but the object glass "full of light." Then when a plate is properly exposed and developed, the deepest shadows come out as clear as the glass itself, and the negative prints brightly and quickly.

When working with 3", 2", or even 1" objectives, the fine adjustment may be dispensed with, and then, if the microscope be of the old Ross pattern, the tube may be entirely discarded, and the objective screwed (by means of a short adapter, if need be, or simply wedged) into the arm that usually carries the tube. By this means the field is only limited by the aperture of the objective, and there is no possibility of flare from the tubes.

A mechanical stage, with concentric rotation, will greatly facilitate the adjustment of the picture in the centre of the plate. With objectives of higher power than $\frac{1}{4}$", these mechanical motions become indispensable. Spring clips are required to keep the object in a vertical position, but elastic bands make good substitutes.

The camera may be of a size to take plates from $\frac{1}{4}$ to whole plate size according to the worker's choice, but at first, at all events, if not permanently, the smaller sizes are best. The quarter plate size (*i.e.*, $4\frac{1}{4}$"

× 3¼″) is most generally used, and plates of that size are always obtainable and are cheap, but we would recommend 5″ × 5″ as a more useful size, conforming more nearly to the circular form of the field, and to many objects, diatoms, echimus spines, sections of stems, &c., which involve a great waste of surface when taken on oblong plates. By means of a simple adapter a camera of this size will take ¼ plates when desired, and these can be placed with their longer edges either horizontal or vertical.

The camera should be made with a bellows body capable of closing up to 6 or 7 inches and extending to about a yard, it will then be available for use, either with or without the eye-piece. To the front should be fitted the base of a smaller bellows of conical form, whose small end terminates in a wood, metal or cardboard ring, lined with black velvet and fitting smoothly and light tight on to the outside of the tube of the microscope. The dark slide which carries the plate should be single, and constructed, to take wet plates as well as dry. Unless a very large camera or a very small microscope is used, the camera will probably want blocking up to bring its centre exactly to the same height as the optic axis of the microscope. For this purpose a light support of pine should be constructed. It should be as long as the camera when fully extended and should be graduated in inches along one edge. If polished it will keep cleaner and look much better than if left plain, and will not wear the edges of the bellows. On the top of this support the two ends of the camera may be made to slide, and may be secured in any position by set screws running in a central groove.

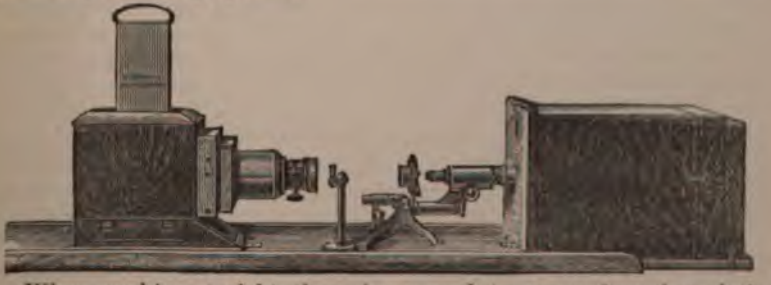
The microscope, camera, illuminating apparatus, &c., are to be clamped down in marked positions on a base board, about six feet long, 11 inches wide, and one inch thick. The point of the base board, immediately below the position of the object when in use, should be marked, and the board should be graduated in inches from this point in both directions. The camera support should have a play of five or six inches to, or from the, microscope, and when adjusted, should be clamped by a single turn of each of two screws, which hold it to the base board. To meet the wants of those who are content to use the eye-piece, Mr. Stanley, of London Bridge, has constructed a cheap apparatus, consisting of an ordinary ¼-plate bellows, camera, and a base board, to carry microscope and lamp, and with adjustable platform to bring the camera up to the height of any ordinary microscope.[1]

As to illumination, there is a great choice, but we may at once dismiss for ordinary work; *Sunlight*, as being too precarious, and necessitating mid-day leisure; *Magnesium*, as too expensive, and very difficult to focus by, on account of its trick of going out when left to itself. *Electric arc* necessitates the charging and discharging of 30 or 40 Grove's cells, a pleasure that can be appreciated only by those who have tried it. In favour of *Incandescent lamps* we cannot say a word. A 20-candle

[1]Mr. Stanley also publishes a pamphlet, containing instructions for working dry plates, silver printing, &c., which is worthy the attention of *quite* beginners.

Swan lamp requires as powerful a battery as a small arc, and has none of its advantages. Its light is not concentrated, it is feeble, and it is yellow. *Lime light* is the cheapest, least troublesome, and, on the whole, the best of powerful artificial illuminants, but still the manufacture of the oxygen and the filling of the bags make a large hole in the evening's work. So, then, our choice is limited to gas and paraffin. Of these, the latter is everywhere obtainable, gives the whiter light, and is all that could be desired for ordinary work with objectives up to $\frac{1}{8}$". Our own arrangement consists of a paraffin lamp with $1\frac{1}{2}$" wick placed close to one end of the oblong zinc reservoir. It is supported on a block about 20 inches from the stage. The glass chimney is narrow to allow of the close approximation of a plano-convex condensing lens of 3" aperture and 3" focus, which collects the light and transmits it in a slightly convergent beam. At a distance of 5 inches from the stage is placed a second plano-convex lens of 3" aperture and 5" focus. This further converges the beam on the object, and gives a brightly and uniformly illuminated disc of about $\frac{3}{4}$ of an inch in diameter, so that quite large objects can be well photographed under low powers. The object of using a short focus lens near the lamp is, of course, to intercept as large a quantity of light as possible, while the longer focus lens nearer the stage secures a larger disc than would be possible with a lens of shorter focus. The convex sides of both the lenses are turned towards each other. When working with the $\frac{1}{2}$ inch and higher powers, a further concentration of the light is affected by an achromatic condenser. This is nothing but a dividing French triplet object glass of about $\frac{1}{4}$" focus. If the edge of the flame is turned towards the condensers, the light is very bright, but there is a difficulty in illuminating a large field uniformly. If, however, the flame make an angle of about 5 degrees with the optic axis, this difficulty disappears without sensibly reducing the brightness of the field.

Mr. G. E. Davis whom we have to thank for the loan of the woodcut, uses the arrangement there figured, in which an optical lantern replaces the simple lamp, with the result of shortening exposures, and facilitating focussing under high powers.



When working at night, there is no need to use a focussing cloth. The image is first received on a screen of the finest possible ground-glass, made more transparent by waxing the ground surface, but even this is too coarse for delicate focussing, and to get the final focus the

screen is removed and replaced by a lens of about 1" focus held in the hand, but attached to a strip of wood, whose arms are brought up against the end of the camera when in use, so that, in shifting it about to examine different parts of the image, it is constrained to remain in one plane, and its distance has been so adjusted once and for all, by previously focussing the ground-glass when in position, that its focal plane is exactly that occupied by the sensitive plate. Mr. Stanley's arrangement for focussing consists of an ordinary ground-glass screen, with cover glasses cemented on to the ground side here and there, so that at these places the screen becomes almost as clear as plain glass, and parts of the image can be examined through them with an ordinary focussing lens. When the apparatus is used without the eye-piece, focussing is effected by turning a rod running underneath the camera, and actuating a roller round which, and also round a groove in the fine adjustment screw above, an elastic band passes.

The possible non-coincidence of the visual and actinic foci of the object glasses, is a point that must be borne in mind, and each objective must be tested to ascertain to what extent, if at all, this non-coincidence occurs. This source of error is, however, usually eliminated by interposing, in the path of the beam between the two condensers when focussing, a glass cell with parallel sides, containing a solution of cuperic ammonic-sulphate[1]. This will cut off all the rays of low refrangibility, and in 9 object glasses out of 10, will be found to supersede any other correction, at all events, when paraffin is the illuminant. With the electric arc or other light in which the ultraviolet rays largely predominate, the case is different. The mode of testing for this want of coincidence, and ascertaining its amount is to interpose the "copper" cell and obtain the best possible visual focus of some object with hard and sharp lines, with the glass to be tested. The cell is then removed, a photograph is taken, and if it shews any want of sharpness, the cell is again interposed, and by means of the fine adjustment screw, the object glass is approached a little nearer to the object, until the image as seen with the eye is about as indistinct as that on the photograph. Another plate is then exposed and developed, and will probably be quite sharp. If not, another slight alteration of focus must be made, and the process repeated if necessary, until at last the requisite sharpness is obtained. It should then be noticed how much the fine adjustment screw has to be turned, to bring back the proper visual focus, when the copper cell is used, and this amount should be recorded and applied as a constant correction whenever the lens in question is used.

So much for apparatus. Now we must say a few words about the photographic processes employed. Gelatine dry plates are the best suited to this work on account of their rapidity, the little apparatus required, the cleanliness of the process of development, and their being always ready for use. There are many excellent dry plates of various degrees of rapidity, and still more various prices in the market, and it is very much better to buy them than to make them for one's-self. A rapid plate is most suitable, but there is no occasion for excessive

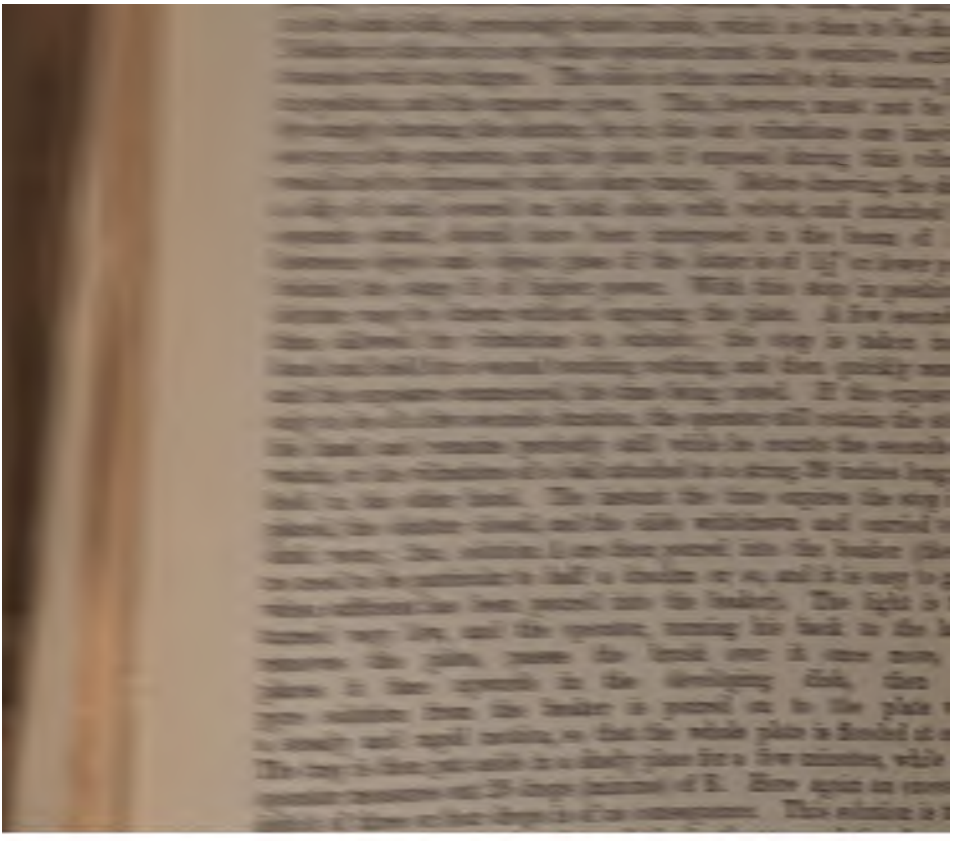
[1] Prepared by adding ammonia to an aqueous solution of sulphate of copper, until the precipitate first formed is re-dissolved.

rapidity. There is no greater mistake than to suppose that 3s. 6d. will purchase a dozen quarter plates of better quality than 2s. or even 1s. 6d. The author now always uses Miall's,[1] though he can speak well of Edwards', the Britannia, Fry's, Lancaster's, and Swan's. Gelatine plates must be exposed to none but deep red light, and the less of this they see the better. When unpacked they should be stored in grooved light-tight boxes. The most tiny ray of white light finding access to the dark room will be fatal to success. If the microscopist works by night only, he will not need a special dark room. Any ordinary room with a closely fitting door to keep out lamp-light or gas light from the adjoining apartment (if any) will answer, provided there is not a lamp within a few yards of the window and the moon is not shining brightly in. The most convenient light to work by is that afforded by a paraffin lamp protected by a chimney of ruby glass, or a screen of good orange demy free from pin holes. This screen must be so arranged as not to allow a single ray of white light to enter the room in any direction, while it allows a proper supply of air to the lamp. If he wishes to work by daylight he must either darken a room or large cupboard entirely, and use the paraffin lamp as before, or he may glaze the window with ruby glass, and make it additionally secure by pasting a layer of yellow demy over it, or two thicknesses of bookbinders' red cloth may be used instead of glass and paper. In whatever way the dark room is lighted, the plate must be shaded from it as much as possible, and only brought into the full light for any length of time when development is nearly complete, and it is necessary to use all safe light to watch the completion of the process. The unpacking and storing of plates, and placing them in the dark slide, may be conducted in almost entire darkness.

Before commencing work the following solutions should have been made up :—

For developing.	A	Pyrogallic acid.....	36 grains	Normal developmer.
		Distilled water	12 oz.	
	B	Ammonia solution ...	½ oz.	
		Potassic Bromide.....	30 grains	
		Distilled water	6 oz.	
	C	Potassic Bromide.....	15 grains	For over-exposure.
		Distilled water	1 oz.	
	D	Ammonia solution ...	1 drm.	For under-exposure.
		Distilled water	1 oz.	
For hardening.		Alum saturated solution in common water.		
For fixing.		Sodic hyposulphite, 4oz. Common water, 1 pint.		
For intensifying.		Mercuric chloride saturated solution in common water. Ammonia solution, 1oz. Common water, 8oz.		
For removing stains		Hydrochloric acid, 8 drops. Common water, 4oz.		

[1]Agent: Fallowfield, Lower Marsh, Lambeth.



By this time the picture should be full of detail, and more light will be required, and may be safely used. This is a most important time, and great judgment is required to know when to stop the development. In the feeble light employed, it will seem as if the picture is blackening all over and disappearing, but really, if properly exposed, it is only gaining density. The judgment will be assisted by removing the plate from the tray, and holding it up before the light; the amount of density can then be better seen. If judged insufficient, the plate is returned to the tray and rocked a few seconds longer. If the exposure has been properly timed, the whole development will occupy about 2½ or 3 minutes from the first application of the B solution. When at last the proper moment arrives, the plate is to be removed from the tray, and well swilled with water from a jug or tap. It is then to be placed face upwards in the dish of hypo, and left there five minutes or so, until all trace of milkiness disappears from the back of the plate. It may then be removed from the hypo, again swilled with water, and if the weather is not hot and the plates are known to have no tendency to frill, it may be placed in a dish of water and left to soak. This soaking must be continued about six hours, in several changes of water. The plate may then be stood on end to dry. Heat must not be applied to hasten the drying, or it will cause the gelatine to dissolve. From the time the plate comes from the hypo it is insensitive to light, and, indeed, it may be taken into the light and fixed there as soon as the developer is washed off, without any considerable harm happening to it, but it is best not to expose it to light until it is fixed. The photographer cannot be sure of the character of his negative until it is fixed, and during this process it will undergo a remarkable change, it will lose its translucency and become transparent. If the exposure and development have been correct, the highest lights will be so dark as to make it very difficult to distinguish even bright objects through them, while the deepest shadows should be almost as clear as the plain glass, and the half tones full of detail.

If on the first application of B, the details come up with undue rapidity and then begin to fade away, the plate has been over-exposed, and the developer must be instantly poured back into the beaker and the plate flooded with water while 20 to 30 drops of C, according to the amount of over-exposure, are measured out and added to the contents of the beaker, which are then to be returned to the plate after the water has been poured off. This will retard the development and preserve the purity of the shadows while the high lights gain in density. After a time 30 or 40 drops of B may be added to complete the development. Under-exposure is indicated by slowness in the appearance of detail in the shadows and half tones after the full quantity of B has been added, and is remedied by the addition of 10 to 20 drops of D to the developer. In all cases when additions have to be made to the developer the additional substance should be poured into the beaker, and the solution from the plate added to it. This will secure a uniform mixture before the developer is returned to the plate. If the additions were simply poured directly into the tray they would act locally and spoil the picture. The

operator must learn to recognize an under or an over-exposed plate by its character after development as well as before. Over exposure results in thin (*i.e.*, weak or transparent) flat, soft negatives, full of detail but deficient of intensity, while an under-exposed and forced plate yields a dense and harsh negative with little detail. It is useful to know this, because one may occasionally wish to give the negative a soft or a harsh character to suit some special subject. The rule is, for violent contrasts, under-expose and force in development; for a finely-graduated image over-expose, and use the bromide freely. For most purposes one should avoid either extreme.

If the films have a tendency to frill (*i.e.*, to pucker and leave the glass at the edges) or blister during or after development, and in very hot weather all plates have, they must be soaked about five minutes in the alum solution, both before and after fixing, to harden the films. In hot weather one should not wait for the commencement of frilling or blistering before applying the remedy, but take it for granted that it will occur, and pass every plate through the alum. It can do no harm in any case.

Plates developed with "pryro" acquire a pale brown or yellow colour, which, if strongly marked, it may be advisable to remove, by a few minutes' immersion in the hydrochloric acid solution. Over-exposed or under-developed plates can be intensified as follows. The mercuric chloride solution is first applied and kept in motion until the negative becomes a very pale grey, almost white. If the operation be conducted in a black dish the negative will appear as a most beautiful positive when the whitening is complete. The plate should then be thoroughly washed for at least half-an-hour in several changes of water. The weak ammonia solution should then be applied, and, in a few seconds, will blacken the image. It will then want a thorough washing in several changes of water, prolonged through about six hours, after which it may be stood in a rack to dry.

When dry, the negatives must be varnished with photographic varnish, flowed over them while hot. This will protect the films from mechanical injury and from silver stains in printing. When negatives have been intensified, it is especially necessary to varnish them as soon as possible, for they are liable to turn white by exposure to the air.

It is very difficult to lay down any general rule for length of exposure, since there are so many variables—thickness and colour of the object, colour of the medium (balsam especially sometimes acquires a yellow colour highly objectionable), focal length and angular aperture of the object glasses, and the number of lenses and kind of glass of which they are composed, length of camera, rapidity of plates, presence or absence of eye-piece, and mode of illumination, all have to be taken into account, to give some idea of what may be expected, we append particulars of a few of our negatives. The first three were taken with an A eyepiece, and a lamp having a 1″ wick only. The plate was about 12″ from the object.

"*Tongue*" *of Blowfly. Balsam.*—2″ objective by Stanley. Home made plate. 5min. Ferrous oxalate developer. Under-exposed.

Diatoms. Coscinodiscus Excavata.—$\frac{1}{2}$″ objective and achromatic condenser. Home made plate. 10min. Pyro. Correct exposure.

Diatom. Auliscus sculptus.—$\frac{1}{8}$″ objective by Beck, and achromatic condenser. Edwards' plate. 26min. Pyro. Rather over-exposed.

The following were taken without an eye-piece, and with a lamp having a $1\frac{1}{2}$″ wick. The distance of the plate from the object is given in each case.

Tongue of cat injected carmine and stained. Transverse Section. Balsam.—3″ objective of 12° by Browning. Distance $12\frac{3}{4}$″. Miall's plate. 10secs. Pyro. Correct exposure.

Rachis of Pteris Aquilina. Transverse Section unstained. Gly jelly.—2″ objective by Stanley. Distance $22\frac{1}{2}$″. Miall's plate. 1min. Pyro. Over-exposed.

Human Kidney injected carmine. Transverse Section in Balsam.—Object yellowish. 1″ objective by Stanley. Distance 36″. "German" plate. $1\frac{1}{2}$min. Oxalate. Correct exposure.

"*Tongue*" *of Blowfly. Balsam.*—$\frac{1}{4}$″ objective and achr. condenser. Distance 40″. Lancaster's plate. $1\frac{1}{2}$min. Oxalate. Correct exposure.

Spicules of Synapta.—Dark ground illumination by spot lens. 1″ objective by Stanley. Distance 41″. "German" plate. 2min. Oxalate. Correct exposure.

Silicine.—Polarized light. Crossed nicols. Without selenite. 1″ objective. Distance 20″. Home made plate. $2\frac{1}{2}$min. Oxalate. Correct exposure.

Ctenoid scales. Skin of sole. Dry, as opaque object.—White, but under cover-glass, and therefore loss of light by reflection. Light condensed as usual, but incident at about 10°. 3″ objective of 12° by Browning. Distance 28″. Miall's plate. 5min. Pyro. Correct exposure.

These examples have been selected as illustrative of very various objects, magnifying powers and modes of illumination. Opaque objects are the most difficult on account of the trouble of focussing by the small amount of light they reflect.

Although any object glass may be used for this work, some possess qualities which specially fit them for it, and there are others in the same degree unsuitable. The most important properties of an objective for photographic work—good definition being, of course, pre-supposed as essential for all purposes—are penetration and flatness of field. These qualities are of greater importance in lenses for photographic than for visual purposes, for in viewing an object under the microscope the observer has the power of focussing in rapid succession, and by imperceptible gradations, points at different depths and different distances from the centre of the field; but a photograph represents only such structures as were in focus at the time of exposure, and once taken, the focus is unalterable. It is, therefore, desirable to secure as great a depth of focus and as flat a field as possible—qualities which are incompatible with large apertures.

APPENDIX.

SECTION IV.

THE METHODS OF MICROSCOPICAL RESEARCH.

PAGE 13.—l. 4 from foot, for "bacteriod" read "bacteroid."

PAGE 20.—l. 19 from top, for "as *a* permanent specimens" read "as permanent specimens."

PAGE 21.—l. 14 from foot, for "clearings" read "clearing."

PAGE 21.—l. 7 from foot, for "choral" read "chloral."

PAGE 23.—l. 7 from foot, for "epithelium for" read "epithelium from."

PAGE 43.—l. 20 from top, for "mythelated" read "methylated."

PAGE 49.—l. 11 from top, for "purist" read "purest."

PAGE 55.—Foot Note, for "Diatomes" read "Diatoms."

PAGE 59.—l. 13 from foot, for "from two or three days" read "from two to three days."

PAGE 69.—l. 20 from top, for "Lieburkuhn" read "Lieberkuhn."

PAGE 69.—l. 2 from bottom (foot note), for "transmitted lights" read "transmitted light."

PAGE 70.—l. 18 from top, for "complimentary" read "complementary."

PAGE 72.—l. 24 from top, for "analine" read "aniline."

PAGE 72.—l. 29 from top, for "analine blue" read "aniline blue."

PAGE 79.—l. 18 from bottom, for "normal developmer" read "normal developer."

PAGE 80.—l. 2 from top, also Line 4 from top, for "papier-machie" read "papier maché."

PAGE 82.—l. 19 from top, for "pryro" read "pyro."

PAGE 83.—l. 1 from top, for "Coscinodiscus excavata" read "Coscinodiscus excavatus."

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THE METHODS OF MICROSCOPICAL RESEARCH.

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